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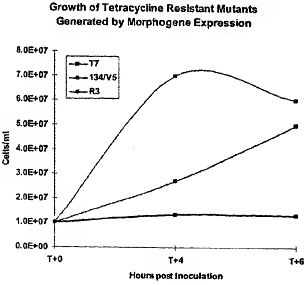
(54) Title: METHODS FOR GENERATING ANTIBIOTIC RESISTANT MICROBES AND NOVEL ANTIBIOTICS



(57) Abstract: The invention provides methods for generating antibiotic resistant bacteria comprising blocking mismatch repair in a bacterium to make hypermutable bacteria, contacting the bacteria with at least one antibiotic, selecting bacteria that are resistant to the antibiotic, and culturing the antibiotic resistant bacteria. The invention also provides methods of determining the genes responsible for antibiotic resistance.

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METHODS FOR GENERATING ANTIBIOTIC RESISTANT MICROBES AND NOVEL ANTIBIOTICS

FIELD OF THE INVENTION

This invention relates to the field of antimicrobial treatments and gene targets for the discovery of antimicrobial agents. In particular, it relates to the discovery of genes essential for growth and virulence of bacteria.

BACKGROUND OF THE INVENTION

Despite the development of new classes of antimicrobial agents over the past decade (reviewed in http://vet.purdue.edu/bms), microbial infections remain a serious health problem. While antibiotics treatment has been effective in controlling infectious diseases, an increase in the number of antibiotic-resistant (AR) microbes have emerged and are now posing a major therapeutic problem. In today's industrialized societies, infectious strains can be found that are resistant to all classes of antimicrobial agents used in the clinic. Infections due to resistant strains include higher morbidity and mortality, longer patient hospitalization, and an increase in treatment costs (Murray (1994) *New Engl. J. Med.* 330:1229-1230). In light of these findings, an unmet need exists for the development of new therapeutic agents that can work by inhibiting the ever-increasing number of novel antibiotic resistance mechanisms.

One approach for generating new therapies and/or therapeutic strategies against AR microbes is to develop methods that can generate a wide array of genomic alterations in a microbe's genome that can yield maximal number altered target genes that are capable of eliciting antibiotic resistance. Once an AR strain is developed, it can be used for rapid genome analysis to identify mutant gene(s) that are capable of rendering a microbe resistant to an antibiotic for target identification. Such analysis can involve any of a variety of methods used by those skilled in the art for identifying mutations and/or differential gene expression,

including but not limited to differential gene expression using microarrays, cDNA subtraction, differential protein analysis, complementation assays, single nucleotide polymorphosm (SNP) analysis or whole genome sequencing to identify altered loci.

A bottleneck to generating genetically diverse microbes is the inability to generate nonbiased genome-wide mutations. Many mutagenesis methods are available whereby the use of chemical and radiation exposure has been successful in generating genomic mutations. A limitation of this approach is that these various methods are usually DNA site specific or are extremely toxic, therefore limiting the mutation spectra and the opportunity to identify a maximal number of genes, when mutated, that are able to confer resistance to an antibiotic. Recently, work done by Nicolaides, *et al.* (Nicolaides *et al.* (1998) *Mol. Cell. Biol.* 18:1635-1641; U.S. patent 6,146,894) has demonstrated the utility of introducing dominant negative mismatch repair mutants into cells to confer global DNA hypermutability. These mutations are in the form of point mutations or small insertion-deletions that are distributed equally throughout the genome. The ability to manipulate the mismatch repair (MMR) process of a target host organism can lead to an increase in the mutability of the target host genome, leading to the generation of innovative cell subtypes with varying phenotypes from the original wild-type cells. Variants can be placed under a specified, desired selective process the result of which is the capacity to select for a novel organism that expresses an altered biological molecule(s) and has a new phenotype. The concept of creating and introducing dominant negative allele of a gene, including the MMR alleles, in bacterial cells has been documented to result in genetically altered prokaryotic mismatch repair genes (Aronshtam and Marinus (1996) *Nucl. Acids Res.* 24:2498-2504; Wu and Marinus (1994) *J. Bacteriol.* 176:5393-400; Brosh and Matson (1995) *J. Bacteriol.* 177:5612-5621). Furthermore, altered MMR activity has been demonstrated when MMR genes from different species including yeast and mammalian cells are over-expressed (Fishel *et al.* (1993) *Cell* 7:1027-1038; Lipkin *et al.* (2000) *Nat. Genet.* 24:27-35). The ability to create hypermutable organisms by blocking MMR has great commercial value for the generation of AR bacterial strains for drug screening and target discovery.

There is an urgent need in the art to elucidate the mechanisms of antimicrobial resistance, and to identify novel antimicrobial agents.

SUMMARY

The invention provides new uses of MMR deficiency in bacteria to identify antibiotic resistance (AR) genes and pathways that can lead to the generation of novel therapeutic strategies for alternative clinical strategies.

Antibiotic resistant (AR) microbes express a number of genes that are essential for growth of the organism in an infection, and serve as useful reagents for target discovery and/or screening lines for the discovery of novel antimicrobial agents. This invention provides an approach to the identification of genes that confer anti-microbial resistance, and the use of those genes, and bacterial strains expressing mutant forms of genes, in the identification, characterization, and evaluation of targets for therapeutic development. In addition, this application teaches of the use of employing structural information of the gene, gene product and mutant strains in screening for antimicrobial agents active against the genes and their corresponding products and pathways. Positive compounds can then be used as final products or precursors to be further developed into antibacterial agents. This invention also provides methods of treating microbial infections in mammals by administering an antimicrobial agent active against such an identified target gene or product, and the pharmaceutical compositions effective for such treatment.

To identify genes capable of rendering bacteria antibiotic-resistant, the invention provides methods of decreasing MMR activity of a microbial host to produce AR strains. Using this process, commercially viable microbes that are resistant to a wide range of antibiotics can be directly selected for the resistance to an anti-microbial agent of interest. AR microbes may be genetically screened to identify novel therapeutic targets for drug develoment. Once a bacterium with a specified resistance is isolated, the MMR activity may be restored by several methods well known to those skilled in the art as a means to gentically "fix" the new mutations in the host genome, thereby making the AR microbe suitable for comparative molecular analysis to the wild-type strain as well as for drug screening to identify novel antimicrobial compounds. For example, if MMR is decreased by the use of a dominant-negative allele or antisense vector directed to an internal MMR gene, the endogenous repair activity can be restored if the gene is expressed by an inducible promoter system, including but not limited to promoters such as: TAC-LACI, tryp (Brosius *et al.* (1984) *Gene* 27:161-172), araBAD (Guzman *et al.* (1995) *J. Bact.* 177:4121-4130) pLex (La Vallie *et al.* (1992) *Bio.Technology* 11:187-193), pRSET (Schoepfer, R. (1993) *Gene* 124:83-85), pT7 (Studier

(1991) *J. Mol. Biol.* 219(1):37-44) *etc.*, by removing the inducer and, therefore, reducing the the promoter activity. In the case that the expression vector employs a Cre-lox system, MMR can be restored by disrupting the cDNA gene insert from the host cell harboring the expression vector (Hasan, N. *et al.* (1994) *Gene* 2:51-56). Yet other methods include homologous knockout of the expression vector that can turn off the actively expressed gene used to inhibit MMR activity. In addition to the recombinant methods outlined above that have the capacity to eliminate the MMR activity from the microbe, it has been demonstrated that many chemicals have the ability to "cure" microbial cells of plasmids. For example, chemical treatment of cells with drugs including bleomycin (Attfield *et al.* (1985) *Antimicrob. Agents Chemother.* 27:985-988) or novobiocin, coumercycin, and quinolones (Fu *et al.* (1988) *Chemotherapy* 34:415-418) have been shown to result in microbial cells that lack endogenous plasmid as evidenced by Southern analysis of cured cells as well as sensitivity to the appropriate antibiotic (Attfield *et al.* (1985) *Antimicrob. Agents Chemother.* 27(6):985-988, Fu *et al.* (1988) *Chem. Abstracts* 34(5):415-418; BiWang *et al.* (1999) *J. of Fujian Agricultural University* 28(1):43-46; Brosius, J. (1988) *Biotechnology* 10:205-225). Whether by use of recombinant means or treatment of cells with chemicals, removal of the MMR-expression plasmid results in the reestablishment of a genetically stable microbial cell line. Therefore, the restoration of MMR allows host bacteria to function normally to repair DNA. The newly generated mutant bacterial strain that exhibits a novel anti-microbial resistance is now suitable for gene/protein discovery to identify new biomolecules that are involved in generating resistance as well as a model system to screen for novel anti-microbial agents targeted against certain antibiotic resistant strains.

In certain embodiments, the invention provides methods for generating antibiotic resistant bacteria comprising the steps of:

blocking mismatch repair in the bacterium whereby the bacterium becomes hypermutable;

contacting the bacterium with at least one antibiotic

determining whether the bacterium is resistant to the antibiotic, thereby generating antibiotic resistant bacteria.

In the methods of the invention, mismatch repair may be blocked in some embodiments by introducing a polynucleotide encoding a wild-type allele of a mismatch repair gene into a cell, whereby the wild-type allele inactivates the endogenous MMR activity by

binding to and interfering with the resident activity. The cell becomes hypermutable as a result of the introduction of the gene.

In other embodiments of the invention, a polynucleotide encoding a dominant negative allele of a mismatch repair gene is introduced into a cell, where the dominant negative gene is derived from a mismatch repair gene from a different organism. The cell becomes hypermutable as a result of the introduction of the gene. In particular embodiments of this method, MMR activity is inhibited for ten rounds of cell division and then the MMR activity is restored therefore restoring the genetic stability. An example of a dominant negative MMR gene is the *PMS2-134* gene.

In other embodiments of the invention, a polynucleotide encoding an allele of a mismatch repair gene is introduced into a bacterial cell, where the mismatch repair gene is derived from a wild-type or altered mammalian, yeast, fungal, amphibian, insect, plant or bacterial mismatch repair gene. The cell becomes hypermutable as a result of the introduction of the gene.

In another embodiment, mismatch repair may be blocked by introducing an antisense nucleic acid molecule into the bacterium wherein the antisense nucleic acid molecule specifically binds to a mismatch repair gene and inhibits mismatch repair in the bacterium.

In other embodiments of the invention, methods are provided for generating a genetic alteration of a bacterial host genome to produce variant strains expressing new output traits. Transgenic bacterium comprising a polynucleotide encoding a wild-type allele of a mismatch repair gene is grown. The bacteria are comprised of a set of altered genes for a desired biological phenotype.

In other embodiments of the invention, methods are provided for generating a genetic alteration of a bacterial host genome to produce variant strains that are resistant to antimicrobial agents. Bacteria with decreased mismatch repair are grown. The bacteria are comprised of a set of altered genes for a desired antibiotic-resistance phenotype.

In further embodiments of the invention, methods are provided for creating a hypermutable bacterium using a wild-type MMR allele for antibiotic-resistance selection, and restoring genomic stability of a selected host by inactivating or decreasing the expression of the wild-type MMR allele.

In another embodiment of the invention, a method is provided for creating a hypermutable bacteria using a dominant negative MMR allele for antibiotic-resistance

selection, and restoring genomic stability of a selected host by inactivating or decreasing the expression of the dominant negative MMR gene allele.

In another embodiment of the invention, a method is provided for creating a hypermutable bacteria expressing an antisense gene to a MMR gene for antibiotic-resistance selection, and restoring genomic stability of a selected host by inactivating or decreasing the expression of the dominant negative MMR gene allele.

In another embodiment of the invention, a method is provided for creating a hypermutable bacteria using chemical inhibitors of MMR for antibiotic-resistance selection, and restoring genomic stability of a selected host by removing the chemical inhibitor. by introducing a dominant negative allele of a mismatch repair gene into the bacterium. The dominant negative allele may be, for example, a *PMS2*-134 gene.

In another embodiment, mismatch repair may be blocked by exposing the bacterium a to a compound that inhibits mismatch repair whereby cells are grown in the presence of the compound and undergo multiple rounds of cell divison in the absence of MMR, yielding sibs that are genetically diverse. Sibs are then selected for antibiotic resistance. AR strains are removed from chemical inhibitor and the endogenous MMR activity is restored leaving genetically stable strains that are now suitable for gene discovery and/or therapeutic agent development. For example, the compound that blocks mismatch repair may be an anthracene derivative, including, but not limited 1,2-dimethylanthracene, 9,10-dimethyl anthracene, 7,8-dimethylanthracene, 9,10-diphenylanthracene, 9,10-dihydroxymethylanthracene, 9-hydroxymethyl-10-methylanthracene, dimethylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-3,4-diol, 9, 10-di-m-tolyanthracene. In other embodiments, the compound that blocks MMR activity is an ATP analog. In other embodiments, the compound that blocks MMR activity is a nuclease inhibitor. In other embodiments, the compound that blocks MMR activity is a DNA polymerase inhibitor.

The methods of the invention may further comprise exposing the bacteria to chemical mutagens. While it has been documented that MMR deficiency can lead to as much as a 1000-fold increase in the endogenous DNA mutation rate of a host, there is no assurance that MMR deficiency alone will be sufficient to alter every gene within the DNA of the host bacterium to create altered biochemicals with new activity(s). Therefore, the use of chemical agents and their respective analogues such as methane sulfonate, dimethyl sulfonate, O-6-methyl

benzadine, ethylnitrosourea (ENU), ethidium bromide, ethyl methanesulfonate (EMS), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), methylnitrosourea (MNU), Tamoxifen, 8-hydroxyguanine, as well as others listed but not limited to in publications by: Khromov-Borisov *et al.* (1999) *Mutat. Res.* 430:55-74; Ohe *et al.* (1999) *Mutat. Res.* 429:189-199; Hour *et al.* (1999) *Food Chem. Toxicol.* 37:569-579; Hrelia *et al.* (1999) *Chem. Biol. Interact.* 118:99-111; Garganta *et al.* (1999) *Environ. Mol. Mutagen.* 33:75-85; Ukawa-Ishikawa *et al* (1998) *Mutat. Res.* 412:99-107; www.ehs.utah.edu/ohh/mutagens, *etc.* can be used in the methods of the invention to further enhance the spectrum of mutations and increase the likelihood of obtaining alterations in one or more genes that can in turn generate host bacteria with a complex antibiotic resistant phenotype (Fu *et al.* (1988) *Chemotherapy* 34(5):415-418; Lee *et al.* (1994) *Mutagenesis* 9:401-405; Vidal *et al.* (1995) *Carcinogenesis* 16:817-821). Prior art teaches us that mismatch repair deficiency leads to hosts with an increased resistance to toxicity by chemicals with DNA damaging activity. This feature allows for the creation of additional genetically diverse hosts when MMR defective bacteria are exposed to such agents, which would be otherwise impossible due to the toxic effects of such chemical mutagens (Colella *et al.* (1999) *Br. J. Cancer* 80:338-343; Moreland *et al.* (1999) *Cancer Res.* 59:2102-2106; Humbert *et al.* (1999) *Carcinogenesis* 20:205-214; Glaab *et al.* (1998) *Mutat. Res.* 398:197-207). Moreover, prior art teaches us that MMR is responsible for repairing chemical-induced DNA adducts, so therefore blocking this process could theoretically increase the number, types, mutation rate and genomic alterations of a bacterial host [Rasmussen *et al.* (1996) *Carcinogenesis* 17:2085-2088; Sledziewska-Gojska *et al.* (1997) *Mutat. Res.* 383:31-37; Janion *et al.* (1989) *Mutat. Res.* 210:15-22). In addition to the chemicals listed above, other types of DNA mutagens include ionizing radiation and UV-irradiation, which are known to cause DNA mutagenesis in bacteria can also be used to potentially enhance this process. These agents, which are extremely toxic to host cells and, therefore, result in a decrease in the actual pool size of altered bacterial cells, are more tolerated in MMR defective hosts and in turn allow for a enriched spectrum and degree of genomic mutation (Drummond *et al.* (1996) *J. Biol. Chem.* 271:9645-19648). such as, but not limited to methane sulfonate, dimethyl sulfonate, O-6-methyl benzadine, ethylnitrosourea, ethidium bromide, ethyl methanesulfonate, N-methyl-N'-nitro-N-nitrosoguanidine, methylnitrosourea, Tamoxifen, and 8-hydroxyguanine.

The methods of the invention may be used to generate AR bacteria which are resistant to such antibiotic compounds as, for example, quinilones, aminoglycosides, magainins,

defensins, tetracyclines, beta-lactams, macrolides, lincosamide, sulfonamides, chloramphenicols, nitrofurantoins, and isoniazids.

In the methods of the invention, the step of determining whether the bacterium is resistant to an antibiotic may comprise analyzing the bacterium for multiantiboitic resistance.

Further, the methods of the invention may comprise making antibiotic resistant bacteria genetically stable, such as by removing the MMR inhibitory molecule, for example.

In the methods of the invention, the genome of the antibiotic resistant bacterium and the genome of a wild-type strain of the bacterium may be compared by sequence analysis of the entire genomes, or compared by microarray analysis, for example. In another embodiment, the genome of said antibiotic resistant bacterium and the genome of said wild-type strain of said bacterium are compared by:

introducing gene fragments from the antibiotic resistant bacterium into the wild-type bacterium, thereby producing mutant bacteria;

selecting a mutant bacterium with antibiotic resistance; and sequencing the gene fragment from the mutant bacterium with antibiotic resistance, thereby identifying the antibiotic resistant gene.

The invention also provides methods of using microbial strains that are naturally defective for MMR due to defects in genes encoding for MMR proteins. Strains in which *mutS, mutL, mutH, or mutY* genes are defective have been reported to be defective in MMR activity (Modrich (1994) *Science* 266:1959-1960). The methods of the invention may employ bacterial strains with mutant endogenous MMR genes for selecting for variants that are AR. Once an AR variant strain is identified, the genetic stability of the microbe can be restored by expressing a functional gene that can complement the defective MMR gene activity.

Mutant strains can be used for gene identification by isolating DNA fragments derived from the MMR defective antibiotic-resistant strains. These bacteria contain DNA fragments with altered sequences that can be introduced into wild-type counterparts (antibiotic susceptible) and screened for fragments that confer antibiotic resistance. Conversely, DNA fragments derived from the wild-type bacteria can be introduced into mutant bacterial strains to screen for genes effective via loss-of-function mutated genes. The fact that a clone is complemented suggests the introduced fragment contains a gene encoding for an antibiotic-resistant gene product. Other methods can also be used to identify AR genes including but not limited to microarray analysis of gene expression, differential expression and/or differential

protein analysis know by those skilled in the art.

The microbial strains described herein have either been generated and characterized in a manner which essentially provides a process by which the manipulation of MMR can confer AR against a wide range of anti-microbial compounds and that these strains are now useful for target discovery and/or therapeutic agent discovery as screening lines.

In other embodiments of the invention, methods of producing a stable bacterium expressing a new phenotype is provided. Turning off the expression of the MMR-wild-type alleles, MMR-dominant negative alleles, or MMR-antisense alleles, results in genetically stable bacteria expressing a new output trait(s).

The invention also provides antibiotic resistant strains of bacteria produced by the methods of the invention.

These and other aspects of the invention provide the art with methods that can generate enhanced mutability in bacteria as well as providing prokaryotic organisms harboring potentially useful mutations to generate novel output traits for commercial applications, and are set forth in greater detail below.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 shows growth of tetracyclin–resistant mutant bacteria carrying a dominant negative allele of PMS2 in the pT7Ea plasmid (134/V5), tetracyclin–resistant mutant bacteria carrying a the PMSR3 gene in the pT7Ea plasmid (R3), and wild-type bacteria carrying the empty pT7Ea plasmid (T7), on medium containing tetracyclin at 0, 4 and 6 hours after tetracycline addition.

DETAILED DESCRIPTION OF THE INVENTION

The inventors present a method for developing hypermutable bacteria by altering the activity of endogenous mismatch repair (MMR) activity of hosts to generate antibiotic resistant (AR) microbes for target discovery and the development of novel anti-microbial agent by screening for new compounds. Wild-type and some dominant negative alleles of mismatch repair genes, when introduced and expressed in bacteria, increase the rate of spontaneous mutations by reducing the effectiveness of the endogenous MMR-mediated DNA repair activity, thereby rendering the bacteria highly susceptible to genetic alterations due to hypermutability. Hypermutable bacteria can then be utilized to screen for novel mutations in a

gene or a set of genes that produce variant siblings exhibiting new output traits not found in the wild-type cells such as antibiotic resistance.

The process of mismatch repair, also called mismatch proofreading, is an evolutionarily highly conserved process that is carried out by protein complexes described in cells as disparate as prokaryotic cells such as bacteria to more complex mammalian cells (Modrich (1994) *Science* 266:1959-1960; Strand *et al.* (1993) *Nature* 365:274-276; Su *et al.* (1988) *J. Biol. Chem.* 263:6829-6835; Aronshtam and Marinus (1996) *Nucl. Acids Res.* 24:2498-2504; Wu and Marinus (1994) *J. Bacteriol.* 176:5393-400). A mismatch repair gene is a gene that encodes one of the proteins of such a mismatch repair complex. Although not wanting to be bound by any particular theory of mechanism of action, a mismatch repair complex is believed to detect distortions of the DNA helix resulting from non-complementary pairing of nucleotide bases. The non-complementary base on the newer DNA strand is excised, and the excised base is replaced with the appropriate base that is complementary to the older DNA strand. In this way, cells eliminate many mutations that occur as a result of mistakes in DNA replication, resulting in genetic stability of the sibling cells derived from the parental cell.

Some wild-type MMR gene alleles as well as dominant negative alleles cause a mismatch repair defective phenotype even in the presence of a wild-type MMR gene allele in the same cell. An example of a dominant negative allele of a MMR gene is the human gene *hPMS2-134*, which carries a truncation mutation at codon 134 (Nicolaides *et al.* (1998) *Mol. Cell. Biol.* 18:1635-1641). The mutation causes the product of this gene to abnormally terminate at the position of the 134th amino acid, resulting in a shortened polypeptide containing the N-terminal 133 amino acids. Such a mutation causes an increase in the rate of mutations, which accumulate in cells after DNA replication. Expression of a dominant negative allele of a mismatch repair gene results in impairment of mismatch repair activity, even in the presence of the wild-type allele. Any mismatch repair allele, which produces such effect, can be used in this invention. In addition, the use of over-expressed wild-type MMR gene alleles from human, mouse, plants, and yeast in bacteria has been shown to cause a dominant negative effect on the bacterial hosts MMR activity (Fishel *et al.* (1993) *Cell* 7:1027-1038; Aronshtam and Marinus (1996) *Nucl. Acids Res.* 24:2498-2504; Wu and Marinus (1994) *J. Bacteriol.* 176:5393-400; Lipkin *et al.* (2000) *Nat. Genet.* 24:27-35).

Dominant negative alleles of a mismatch repair gene can be obtained from the cells of humans, animals, yeast, bacteria, plants or other organisms. Screening cells for defective mismatch repair activity can identify such alleles. Mismatch repair genes may be mutant or wild-type. Bacterial host MMR may be mutated or not. The term bacteria used in this application include any organism from the prokaryotic *kingdom.* These organisms include genera such as but not limited to *Agrobacterium, Anaerobacter, Aquabacterium, Azorhizobium, Bacillus, Bradyrhizobium, Cryobacterium, Escherichia, Enterococcus, Heliobacterium, Klebsiella, Lactobacillus, Methanococcus, Methanothermobacter, Micrococcus, Mycobacterium, Oceanomonas, Pseudomonas, Rhizobium, Staphylococcus, Streptococcus, Streptomyces, Thermusaquaticus, Thermaerobacter, Thermobacillus, etc.* Other procaryotes that can be used for this application are listed at (www.bacterio.cict.fr/validgenericnames). Bacteria exposed to chemical mutagens or radiation exposure can be screened for defective mismatch repair. Genomic DNA, cDNA, or mRNA from any cell encoding a mismatch repair protein can be analyzed for variations from the wild-type sequence. Dominant negative alleles of a mismatch repair gene can also be created artificially, for example, by producing variants of the *hPMS2-134* allele or other mismatch repair genes (Nicolaides *et al.* (1998) *Mol. Cell. Biol.* 18:1635-1641). Various techniques of site-directed mutagenesis can be used. The suitability of such alleles, whether natural or artificial, for use in generating hypermutable bacteria can be evaluated by testing the mismatch repair activity (using methods described in Nicolaides *et al.* (1998) *Mol. Cell. Biol.* 18:1635-1641) caused by the allele in the presence of one or more wild-type alleles, to determine if it is a dominant negative allele.

A bacterium that over-expresses a wild-type mismatch repair allele or a dominant negative allele of a mismatch repair gene will become hypermutable. This means that the spontaneous mutation rate of such bacteria is elevated compared to bacteria without such alleles. The degree of elevation of the spontaneous mutation rate can be at least 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, 200-fold, 500-fold, or 1000-fold that of the normal bacteria as measured as a function of bacterial doubling/minute.

According to one aspect of the invention, a polynucleotide encoding either a wild-type or a dominant negative form of a mismatch repair protein is introduced into bacteria. The gene can be any dominant negative allele encoding a protein which is part of a mismatch repair complex, for example, *mutS, mutL, mutH, or mutY* homologs of the bacterial, yeast,

plant or mammalian genes (Modrich (1994) *Science* 266:1959-1960; Prolla *et al.* (1994) *Science* 264:1091-1093). The dominant negative allele can be naturally occurring or made in the laboratory. The polynucleotide can be in the form of genomic DNA, cDNA, RNA, or a chemically synthesized polynucleotide or polypeptide. The molecule can be introduced into the cell by transfection or other methods well described in the literature.

Transfection is any process whereby a polynucleotide or polypeptide is introduced into a cell. The process of transfection can be carried out in a bacterial culture using a suspension culture. The bacteria can be any type classified under the prokaryotes.

In general, transfection will be carried out using a suspension of cells but other methods can also be employed as long as a sufficient fraction of the treated cells incorporate the polynucleotide or polypeptide so as to allow transfected cells to be grown and utilized. The protein product of the polynucleotide may be transiently or stably expressed in the cell. Techniques for transfection are well known to those skilled in the art. Available techniques to introduce a polynucleotide or polypeptide into a prokaryote include but are not limited to electroporation, transduction, cell fusion, the use of chemically competent cells (*e.g.*, calcium chloride), and packaging of the polynucleotide together with lipid for fusion with the cells of interest. Once a cell has been transformed with the inhibitory mismatch repair gene or protein, the cell can be propagated and manipulated in either liquid culture or on a solid agar matrix, such as a petri dish. If the transfected cell is stable, the gene will be retained and expressed at a consistent level when the promoter is constitutively active, or when in the presence of appropriate inducer molecules when the promoter is inducible, for many cell generations, and a stable, hypermutable bacterial strain results.

An isolated bacterial cell is a clone obtained from a pool of a bacterial culture by chemically selecting out strains using antibiotic selection of an expression vector. If the bacterial cell is derived from a single cell, it is defined as a clone.

Bacterial cultures may be screened for antibiotic resistance against a wide array of antibiotic compounds. For example, but not by way of limitation, bacteria produced by the methods of the invention may be screened for resistance to quinilones, aminoglycosides, magainins, defensins, tetracyclines, beta-lactams, macrolides, lincosamide, sulfonamides, chloramphenicols, nitrofurantoins, and isoniazids. The antibiotics may be incorporated into solid or liquid growth medium, for example.

A polynucleotide encoding an inhibitory form of a mismatch repair protein can be introduced into the genome of a bacterium or propagated on an extra-chromosomal plasmid. Selection of clones harboring the mismatch repair gene expression vector can be accomplished by addition of any of several different antibiotics, including but not limited to ampicillin, kanamycin, chloramphenicol, zeocin, and tetracycline. The microbe can be any species for which suitable techniques are available to produce transgenic microorganisms, such as but not limited to *genera* including *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Escherichia* and others.

Any method for making transgenic bacteria known in the art can be used. According to one process of producing a transgenic microorganism, the polynucleotide is transfected into the microbe by one of the methods well known to those in the art. Next, the microbial culture is grown under conditions that select for cells in which the polynucleotide encoding the mismatch repair gene is either incorporated into the host genome as a stable entity or propagated on a self-replicating extra-chromosomal plasmid, and the protein encoded by the polynucleotide fragment transcribed and subsequently translated into a functional protein within the cell. Once a transgenic microbe is engineered to harbor the expression construct, it is then propagated to generate and sustain a culture of transgenic microbes indefinitely.

Once a stable, transgenic microorganism has been engineered to express a functional MMR protein, the microbe can be exploited to create novel mutations in one or more target gene(s) of interest harbored within the same microorganism. A gene of interest can be any gene naturally possessed by the bacterium or one introduced into the bacterial host by standard recombinant DNA techniques. The target gene(s) may be known prior to the selection or unknown. One advantage of employing such transgenic microbes to induce mutations in resident or extra-chromosomal genes within the microbe is that it is unnecessary to expose the microorganism to mutagenic insult, whether it be chemical or radiation in nature, to produce a series of random gene alterations in the target gene(s). This is due to the highly efficient nature and the spectrum of naturally occurring mutations that result as a consequence of the altered mismatch repair process. However, it is possible to increase the spectrum and frequency of mutations by the concomitant use of either chemical and/or radiation together with MMR defective cells. The net effect of the combination treatment is the increase in altered gene pool in the genetically altered microbe that result in an increased alteration of an allele(s) that are useful for producing new output traits. Other benefits of using MMR-defective microbes that are taught in this application are genetic screens for the DIRECT

selection of variant sub-clones that exhibit new output traits with commercially important applications such as antibiotic resistance, which allows the bypassing of the tedious and time consuming gene identification, isolation and characterization stages.

Mutations can be detected by analyzing the recombinant microbe for alterations in the genotype and/or phenotype post-activation of the decreased mismatch repair activity of the transgenic microorganism. Novel genes that produce altered phenotypes in MMR-defective microbial cells can be discerned by any variety of molecular techniques well known to those in the art. For example, the microbial genome can be isolated and a library of restriction fragments cloned into a plasmid vector. The library can be introduced into a "normal" cell and the cells exhibiting the novel phenotype screened. Transformed cells are then screened for the new phenotype (*e.g.*, antibiotic resistance). A plasmid is isolated from those normal, transformed cells that exhibit the novel phenotype and the inserted gene(s) characterized by DNA sequence analysis.

Alternatively, differential messenger RNA screen can be employed utilizing driver and tester RNA (derived from wild-type and novel mutant respectively) followed by cloning the differential transcripts and characterizing them by standard molecular biology methods well known to those skilled in the art. Furthermore, if the mutant sought is on encoded by an extrachromosmal plasmid, then following co-expression of the dominant negative MMR gene and the gene of interest to be altered and phenotypic selection, the plasmid is isolated from mutant clones and analyzed by DNA sequence analysis by methods well known to those in the art.

In another embodiment, the screening of cells may be performed by microarray analysis. In microarray analysis, microchips containing all or a subset of all expressed bacterial genes may be screened using RNA molecules derived from the wild-type or antibiotic resistant strain whereby RNA derived from one strain is reverse transcribed using FluoroLink Cy3 and the other RNA sample is reverse transcribe-labelled using Cy5 dUTP. Labelled cDNAs from each organism are used to probe the microchip whereby unique message from one source will generate a distinct signal while message expressed from both sources will generate a common fluorescence. Alternatively, microchips containing olignucleotide derived from the wild-type strain can be used to hybridize genomic fragments

from the antibiotic resistant strain to identify fragments containing a mutated gene by loss of hybridization.

Phenotypic screening for output traits in MMR-defective mutants can be by biochemical activity and/or a physical phenotype of the altered gene product. A mutant phenotype can also be detected by identifying alterations in electrophoretic mobility, DNA binding in the case of transcription factors, spectroscopic properties such as IR, CD, X-ray crystallography or high field NMR analysis, or other physical or structural characteristics of a protein encoded by a mutant gene. It is also possible to screen for altered novel function of a protein *in situ*, in isolated form, or in model systems. One can screen for alteration of any property of the microorganism associated with the function of the gene of interest, whether the gene is known prior to the selection or unknown. The aforementioned screening and selection discussion is meant to illustrate the potential means of obtaining novel mutants with commercially valuable output traits.

Plasmid expression vectors that harbor the mismatch repair (MMR) gene inserts can be used in combination with a number of commercially available regulatory sequences to control both the temporal and quantitative biochemical expression level of the dominant negative MMR protein. The regulatory sequences can be comprised of a promoter, enhancer or promoter/enhancer combination and can be inserted either upstream or downstream of the MMR gene to control the expression level. The regulatory promoter sequence can be any of those well known to those in the art, including but not limited to the lacI, tetracycline, tryptophan-inducible, phosphate inducible, T7-polymerase-inducible (Studier *et al.* (1991) *J. Mol. Biol.* 219(1):37-44), and steroid inducible constructs as well as sequences which can result in the excision of the dominant negative mismatch repair gene such as those of the Cre-Lox system. These types of regulatory systems have been listed in scientific publications and are familiar to those skilled in the art.

Once a microorganism with a novel, desired output trait of interest is created, the activity of the aberrant MMR activity is attenuated or eliminated by any of a variety of methods, including removal of the inducer from the culture medium that is responsible for promoter activation, gene disruption of the aberrant MMR gene constructs, electroporation and or chemical curing of the expression plasmids (Brosius(1988) *Biotechnology* 10:205-225;

Wang *et al.* (1999) *J. of Fujian Agricultural University* 28:43-46; Fu *et. al.* (1988) *Chem. Abstracts* 34:415-418). The expression of the dominant negative MMR gene will be turned on to select for hypermutable microbes with new output traits. Next, the expression of the dominant negative dominant negative MMR allele is rapidly turned off to reconstitute a genetically stable strain that produces a new output trait of commercial interest. The resulting microbe is now useful as a stable strain that can be applied to various commercial applications, depending upon the selection process placed upon it.

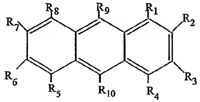
In cases where genetically deficient mismatch repair bacteria (strains such as but not limited to: M1 (mutS) and in EC2416 (mutS delta umuDC), and mutL or mutY strains) are used to derive new output traits, transgenic constructs will be used that express wild-type mismatch repair genes sufficient to complement the genetic defect and therefore restore mismatch repair activity of the host after trait selection (Grzesiuk *et al.* (1988) *Mutagenesis* 13:127-132; Bridges *et al.* (1997) *EMBO J.* 16:3349-3356; LeClerc (1996) *Science* 15:1208-1211; Jaworski, A. *et al.* (1995) *Proc. Natl. Acad. Sci USA* 92:11019-11023). The resulting microbe is genetically stable and can be applied to various commercial practices.

The use of over expressing foreign mismatch repair genes from human and yeast such as human *PMS1* (SEQ ID NO:7), human *PMS2* (SEQ ID NO:5), mouse *PMS2* (SEQ ID NO:3), human *MSH2* (SEQ ID NO:9), human *MLH1* (SEQ ID NO:11), yeast *MLH1* (SEQ ID NO:1), human *MLH3* (SEQ ID NO:28), as well as the other homologs identified in other species for these encoded polypeptides *etc.*have been previously demonstrated to produce a dominant negative mutator phenotype in bacterial hosts (Brosh and Matson (1995) *J. Bacteriol.* 177:5612-5621; Studamire *et al.* (1998) *Mol. Cell. Biol.* 18:7590-7601; Alani *et al.* (1997) *Mol. Cell. Biol.*17:2436-2447). In addition, the use of bacterial strains expressing prokaryotic dominant negative MMR genes as well as hosts that have genomic defects in endogenous MMR proteins have also been previously shown to result in a dominant negative mutator phenotype (Strand *et al.* (1993) *Nature* 365:274-276; Nicolaides *et al.* (1998) *Mol. Cell. Biol.* 18:1635-1641). However, the findings disclosed here teach the use of MMR genes, including the human PMSR2 and PMSR3 gene (Nicolaides *et al.* (1995) *Genomics* 30:195-206); the related *PMS134* truncated MMR gene (Nicolaides *et al.*(1998) *Mol. Cell. Biol.* 18:1635-1641); the plant mismatch repair genes (derived from *Arabidopsis thaliana*),

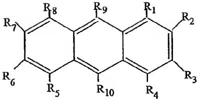
ATPMS2 (SEQ ID NO:30), At *PMS1* (SEQ ID NO:32), and *MutS* homolog (SEQ ID NO:34) and those genes that are homologous to the 134 N-terminal amino acids of the PMS2 gene which include the MutL family of MMR proteins and including the PMSR and PMS2L homologs described by Hori *et al.* (PMS2L8 (SEQ ID NO:36) and PMS2L9 (SEQ ID NO:38)) and Nicolaides (Nicolaides *et al.* (1995) *Genomics* 30:195-206) to create hypermutable microbes. The corresponding polypeptide sequences for the above-referenced nucleic acid sequences are as follows: yeast MLH1 (SEQ ID NO:2); mouse PMS2 (SEQ ID NO:4); human PMS2 (SEQ ID NO:6); human PMS1 (SEQ ID NO:8); human MSH2 (SEQ ID NO:10); human MLH1 (SEQ ID NO:12); PMS2-134 (SEQ ID NO:14); human MSH6 (SEQ ID NO:16); human PMSR2 (SEQ ID NO:18); human PMSR3 (SEQ ID NO:20); human PMSL9 (SEQ ID NO:22); human MLH3 (SEQ ID NO:29); ATPMS2 (SEQ ID NO:31); ATPMS1 (SEQ ID NO:33); At MutS homolog (SEQ ID NO:35); PMS2L8 (SEQ ID NO:37); and PMS2L9 (SEQ ID NO:39).

In addition, the invention provides the use of DNA mutagens in combination with MMR defective microbial hosts to enhance the hypermutable production of genetic alterations. This has not been demonstrated in the art previously as a means to accentuate MMR activity for generation of microorganisms with clinically relevant output traits such as antibiotic resistance.

In some embodiments of the invention, the bacteria cells are rendered hypermutable by introducing a chemical inhibitor of mismatch repair into the growth medium. Chemical inhibitors of mismatch repair that may be used to generate hypermutable bacterial cells include anthracene-derived compounds comprising the formula:



In certain preferred embodiments of the invention, the anthracene has the formula:



wherein R_1-R_{10} are independently hydrogen, hydroxyl, amino, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl,O-alkynyl, S-alkynyl, N-alkynyl, aryl, substituted aryl, aryloxy, substituted aryloxy, heteroaryl, substituted heteroaryl, aralkyloxy, arylalkyl, alkylaryl, alkylaryloxy, arylsulfonyl, alkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl, guanidino, carboxy, an alcohol, an amino acid, sulfonate, alkyl sulfonate, CN, NO_2, an aldehyde group, an ester, an ether, a crown ether, a ketone, an organosulfur compound, an organometallic group, a carboxylic acid, an organosilicon or a carbohydrate that optionally contains one or more alkylated hydroxyl groups;

wherein said heteroalkyl, heteroaryl, and substituted heteroaryl contain at least one heteroatom that is oxygen, sulfur, a metal atom, phosphorus, silicon or nitrogen; and

wherein said substituents of said substituted alkyl, substituted alkenyl, substituted alkynyl,
substituted aryl, and substituted heteroaryl are halogen, CN, NO_2, lower alkyl, aryl, heteroaryl, aralkyl, aralkyloxy, guanidino, alkoxycarbonyl, alkoxy, hydroxy, carboxy and amino;

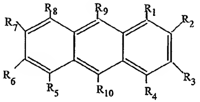
and wherein said amino groups optionally substituted with an acyl group, or 1 to 3 aryl or lower alkyl groups;

or wherein any two of R_1-R_{10} can together form a polyether;

or wherein any two of R_1-R_{10} can, together with the intervening carbon atoms of the anthracene core, form a crown ether.

As used herein, "alkyl" refers to a hydrocarbon containing from 1 to about 20 carbon atoms. Alkyl groups may straight, branched, cyclic, or combinations thereof. Alkyl groups thus include, by way of illustration only, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, cyclopentyl, cyclopentylmethyl, cyclohexyl, cyclohexylmethyl, and the like. Also included within the definition of "alkyl" are fused and/or polycyclic aliphatic cyclic ring systems such

as, for example, adamantane. As used herein the term "alkenyl" denotes an alkyl group having at least one carbon-carbon double bond. As used herein the term "alkynyl" denotes an alkyl group having at least one carbon-carbon triple bond. In certain preferred embodiments of the invention, the anthracene has the formula:



wherein R_1-R_{10} are independently hydrogen, hydroxyl, amino, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl,O-alkynyl, S-alkynyl, N-alkynyl, aryl, substituted aryl, aryloxy, substituted aryloxy, heteroaryl, substituted heteroaryl, aralkyloxy, arylalkyl, alkylaryl, alkylaryloxy, arylsulfonyl, alkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl, guanidino, carboxy, an alcohol, an amino acid, sulfonate, alkyl sulfonate, CN, NO_2, an aldehyde group, an ester, an ether, a crown ether, a ketone, an organosulfur compound, an organometallic group, a carboxylic acid, an organosilicon or a carbohydrate that optionally contains one or more alkylated hydroxyl groups;

wherein said heteroalkyl, heteroaryl, and substituted heteroaryl contain at least one heteroatom that is oxygen, sulfur, a metal atom, phosphorus, silicon or nitrogen; and

wherein said substituents of said substituted alkyl, substituted alkenyl, substituted alkynyl,
substituted aryl, and substituted heteroaryl are halogen, CN, NO_2, lower alkyl, aryl, heteroaryl, aralkyl, aralkyloxy, guanidino, alkoxycarbonyl, alkoxy, hydroxy, carboxy and amino;

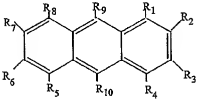
and wherein said amino groups optionally substituted with an acyl group, or 1 to 3 aryl or lower alkyl groups;

or wherein any two of R_1-R_{10} can together form a polyether;

or wherein any two of R_1-R_{10} can, together with the intervening carbon atoms of the anthracene core, form a crown ether.

As used herein, "alkyl" refers to a hydrocarbon containing from 1 to about 20 carbon atoms. Alkyl groups may straight, branched, cyclic, or combinations thereof. Alkyl groups thus include, by way of illustration only, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, cyclopentyl, cyclopentylmethyl, cyclohexyl, cyclohexylmethyl, and the like. Also included within the definition of "alkyl" are fused and/or polycyclic aliphatic cyclic ring systems such as, for example, adamantane. As used herein the term "alkenyl" denotes an alkyl group having at least one carbon-carbon double bond. As used herein the term "alkynyl" denotes an alkyl group having at least one carbon-carbon triple bond.

In some embodiments, the anthracene has the formula:



wherein R_1-R_{10} are independently hydrogen, hydroxyl, amino, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl,O-alkynyl, S-alkynyl, N-alkynyl, aryl, substituted aryl, aryloxy, substituted aryloxy, heteroaryl, substituted heteroaryl, aralkyloxy, arylalkyl, alkylaryl, alkylaryloxy, arylsulfonyl, alkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl, guanidino, carboxy, an alcohol, an amino acid, sulfonate, alkyl sulfonate, CN, NO_2, an aldehyde group, an ester, an ether, a crown ether, a ketone, an organosulfur compound, an organometallic group, a carboxylic acid, an organosilicon or a carbohydrate that optionally contains one or more alkylated hydroxyl groups;

wherein said heteroalkyl, heteroaryl, and substituted heteroaryl contain at least one heteroatom that is oxygen, sulfur, a metal atom, phosphorus, silicon or nitrogen; and

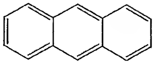
wherein said substituents of said substituted alkyl, substituted alkenyl, substituted alkynyl,

substituted aryl, and substituted heteroaryl are halogen, CN, NO_2, lower alkyl, aryl, heteroaryl, aralkyl, aralkyloxy, guanidino, alkoxycarbonyl, alkoxy, hydroxy, carboxy and amino;

and wherein said amino groups optionally substituted with an acyl group, or 1 to 3 aryl or lower alkyl groups.

Examples of such anthracenes include, but are not limited to 1,2-dimethylanthracene, 9,10-dimethyl anthracene, 7,8-dimethylanthracene, 9,10-diphenylanthracene, 9,10-dihydroxymethylanthracene, 9-hydroxymethyl-10-methylanthracene, dimethylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-3,4-diol, and 9, 10-di-m-tolyanthracene.

As used herein the term "anthracene" refers to the compound anthracene. However, when referred to in the general sense, such as "anthracenes," "an anthracene" or "the anthracene," such terms denote any compound that contains the fused triphenyl core structure of anthracene, i.e.,



regardless of extent of substitution.

In some embodiments, the alkyl, alkenyl, alkynyl, aryl, aryloxy, and heteroaryl substituent groups described above may bear one or more further substituent groups; that is, they may be "substituted". In some preferred embodiments these substituent groups can include halogens (for example fluorine, chlorine, bromine and iodine), CN, NO_2, lower alkyl groups, aryl groups, heteroaryl groups, aralkyl groups, aralkyloxy groups, guanidino, alkoxycarbonyl, alkoxy, hydroxy, carboxy and amino groups. In addition, the alkyl and aryl portions of aralkyloxy, arylalkyl, arylsulfonyl, alkylsulfonyl, alkoxycarbonyl, and aryloxycarbonyl groups also can bear such substituent groups. Thus, by way of example only, substituted alkyl groups include, for example, alkyl groups fluoro-, chloro-, bromo- and iodoalkyl groups, aminoalkyl groups, and hydroxyalkyl groups, such as hydroxymethyl, hydroxyethyl, hydroxypropyl, hydroxybutyl, and the like. In some preferred embodiments such hydroxyalkyl groups contain from 1 to about 20 carbons.

As used herein the term "aryl" means a group having 5 to about 20 carbon atoms and which contains at least one aromatic ring, such as phenyl, biphenyl and naphthyl. Preferred aryl groups include unsubstituted or substituted phenyl and naphthyl groups. The term

"aryloxy" denotes an aryl group that is bound through an oxygen atom, for example a phenoxy group.

In general, the prefix "hetero" denotes the presence of at least one hetero (i.e., non-carbon) atom, which is in some preferred embodiments independently one to three O, N, S, P, Si or metal atoms. Thus, the term "heteroaryl" denotes an aryl group in which one or more ring carbon atom is replaced by such a heteroatom. Preferred heteroaryl groups include pyridyl, pyrimidyl, pyrrolyl, furyl, thienyl, and imidazolyl groups.

The term "aralkyl" (or "arylalkyl") is intended to denote a group having from 6 to 15 carbons, consisting of an alkyl group that bears an aryl group. Examples of aralkyl groups include benzyl, phenethyl, benzhydryl and naphthylmethyl groups.

The term "alkylaryl" (or "alkaryl") is intended to denote a group having from 6 to 15 carbons, consisting of an aryl group that bears an alkyl group. Examples of aralkyl groups include methylphenyl, ethylphenyl and methylnaphthyl groups.

The term "arylsulfonyl" denotes an aryl group attached through a sulfonyl group, for example phenylsulfonyl. The term "alkylsulfonyl" denotes an alkyl group attached through a sulfonyl group, for example methylsulfonyl.

The term "alkoxycarbonyl" denotes a group of formula -C(=O)-O-R where R is alkyl, alkenyl, or alkynyl, where the alkyl, alkenyl, or alkynyl portions thereof can be optionally substituted as described herein.

The term "aryloxycarbonyl" denotes a group of formula -C(=O)-O-R where R is aryl, where the aryl portion thereof can be optionally substituted as described herein.

The terms "arylalkyloxy" or "aralkyloxy" are equivalent, and denote a group of formula $-O-R'-R''$, where R' is R is alkyl, alkenyl, or alkynyl which can be optionally substituted as described herein, and wherein R'' denotes a aryl or substituted aryl group.

The terms "alkylaryloxy" or "alkaryloxy" are equivalent, and denote a group of formula $-O-R'-R''$, where R' is an aryl or substituted aryl group, and R'' is alkyl, alkenyl, or alkynyl which can be optionally substituted as described herein.

As used herein, the term "aldehyde group" denotes a group that bears a moiety of formula -C(=O)-H. The term "ketone" denotes a moiety containing a group of formula -R-C(=O)-R=, where R and R= are independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl,

or alkaryl, each of which may be substituted as described herein.

As used herein, the term "ester" denotes a moiety having a group of formula -R-C(=O)-O-R= or -R-O-C(=O)-R= where R and R= are independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, or alkaryl, each of which may be substituted as described herein.

The term "ether" denotes a moiety having a group of formula -R-O-R= or where R and R= are independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, or alkaryl, each of which may be substituted as described herein.

The term "crown ether" has its usual meaning of a cyclic ether containing several oxygen atoms. As used herein the term "organosulfur compound" denotes aliphatic or aromatic sulfur containing compounds, for example thiols and disulfides. The term "organometallic group" denotes an organic molecule containing at least one metal atom.

The term "organosilicon compound" denotes aliphatic or aromatic silicon containing compounds, for example alkyl and aryl silanes.

The term "carboxylic acid" denotes a moiety having a carboxyl group, other than an amino acid.

As used herein, the term "amino acid" denotes a molecule containing both an amino group and a carboxyl group. In some preferred embodiments, the amino acids are α-, β-, γ- or δ-amino acids, including their stereoisomers and racemates. As used herein the term "L-amino acid" denotes an α-amino acid having the L configuration around the α-carbon, that is, a carboxylic acid of general formula $CH(COOH)(NH_2)$-(side chain), having the L-configuration. The term "D-amino acid" similarly denotes a carboxylic acid of general formula $CH(COOH)(NH_2)$-(side chain), having the D-configuration around the α-carbon. Side chains of L-amino acids include naturally occurring and non-naturally occurring moieties. Non-naturally occurring (i.e., unnatural) amino acid side chains are moieties that are used in place of naturally occurring amino acid side chains in, for example, amino acid analogs. *See*, for example, Lehninger, *Biochemistry*, Second Edition, Worth Publishers, Inc, 1975, pages 72-77, incorporated herein by reference. Amino acid substituents may be attached through their carbonyl groups through the oxygen or carbonyl carbon thereof, or through their amino groups, or through functionalities residing on their side chain portions.

In some embodiments of the methods of the invention, the cells are made hypermutable using ATP analogs capable of blocking ATPase activity required for MMR. MMR reporter cells are screened with ATP compound libraries to identify those compounds capable of blocking MMR *in vivo.* Examples of ATP analogs that are useful in blocking MMR activity include, but are not limited to, nonhydrolyzable forms of ATP such as AMP-PNP and ATP[gamma]S block the MMR activity (Galio, L. *et al.* (1999) *Nucl. Acids Res.* 27:2325-2331; Allen, D.J. *et al.* (1997) *EMBO J.* 16:4467-4476; Bjornson K.P. *et al.* (2000) *Biochem.* 39:3176-3183). The ATPase inhibitors inhibit MMR and the cells become hypermutable as a result.

In other embodiments of the methods of the invention, the cells are made hypermutable using nuclease inhibitors that are able to block the exonuclease activity of the MMR biochemical pathway. MMR reporter cells are screened with nuclease inhibitor compound libraries to identify compounds capable of blocking MMR *in vivo*. Examples of nuclease inhibitors that are useful in blocking MMR activity include, but are not limited to analogs of N-Ethylmaleimide, an endonuclease inhibitor (Huang, Y.C., et.al. (1995) *Arch. Biochem. Biophys.* 316:485), heterodimeric adenine-chain-acridine compounds, exonulcease III inhibitors (Belmont P, et.al., *Bioorg Med Chem Lett* (2000) 10:293-295), as well as antibiotic compounds such as Heliquinomycin, which have helicase inhibitory activity (Chino, M, et.al. *J. Antibiot. (Tokyo)* (1998) 51:480-486). The nuclease inhibitors inhibit MMR and the cells become hypermutable as a result.

In other embodiments of the methods of the invention, the cells are made hypermutable using DNA polymerase inhibitors that are able to block the polymerization required for mismatch-mediated repair. MMR reporter cells are screened with DNA polymerase inhibitor compound libraries to identify those compounds capable of blocking MMR *in vivo*. Examples of DNA polymerase inhibitors that are useful in blocking MMR activity include, but are not limited to, analogs of actinomycin D (Martin, S.J., et.al. (1990) *J. Immunol.* 145:1859), Aphidicolin (Kuwakado, K. et.al. (1993) *Biochem. Pharmacol.* 46:1909) 1-(2'-Deoxy-2'-fluoro-beta-L-arabinofuranosyl)-5-methyluracil (L-FMAU) (Kukhanova M, et.al., *Biochem Pharmacol* (1998) 55:1181-1187), and 2',3'-dideoxyribonucleoside 5'-triphosphates (ddNTPs)

(Ono, K., et.al., *Biomed Pharmacother* (1984) 38:382-389). The polymerase inhibitors inhibit MMR and the cells become hypermutable as a result.

Bacterial cells rendered hypermutable using chemical inhibitors of MMR may be made genetically stable when the desired phenotype is obtained by removing the MMR inhibitory molecule.

In certain embodiments, the bacterial cells are made hypermutable by introducing plamids that generate antisense messages wherein the antisense RNA specifically bind to MMR genes and prevent efficient expression of MMR proteins. Preferably, the antisense transcripts are at least 12 nucleotides in length and, more preferably are at least 20, 30, 40, 50 nucleotides or more in length. The antisense transcripts specifically bind to regions of the MMR gene to inhibit expression. Preferably, the antisense transcripts specifically bind to regulatory regions of the MMR gene such as to the MMR promoter region, Kozak consensus sequences, and the like. As used herein, "specifically bind" refers to association of nucleic acid strands forming complementary base pairing in Watson-Crick arrangement, allowing for mismatches such that 100% complementarity is not required. In general, the complementarity will be about 85%, 90%, 95% or more. Plasmids that may be used to express an antisense MMR transcript include any vector generally known in the art to express antisense transcripts, such as for example, those found in Qian Y. *et al.* (1998) *Mutat. Res.* **418(2-3)**:61-71.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples that will be provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

Example 1: Generation of MMR defective bacteria.

Bacterial expression constructs were prepared using the human PMS2 related gene (hPMSR3) (Nicolaides *et al.* (1995) *Genomics* 30:195-206) and the human PMS134 cDNA (Nicolaides *et al.* (1998) *Mol. Cell. Biol.* 18:1635-1641), both of which are capable of inactivating MMR activity and thereby increase the overall frequency of genomic hypermutation. Moreover, the use of regulatable expression vectors will allow for suppression of dominant negative MMR alleles and restoration of the MMR pathway and

genetic stability in hosts cells (Brosius, J. (1988) *Biotechnology* 10:205-225). For these studies, a plasmid encoding the hPMS134 cDNA was altered by polymerase chain reaction (PCR). The 5' oligonucleotide has the following sequence: 5'-acg cat atg gag cga gct gag agc tcg agt-3' (SEQ ID NO:23)that includes the NdeI restriction site (cat atg). The 3'-oligonucleotide has the following sequence: 5'-gaa ttc tta tca cgt aga atc gag acc gag gag agg gtt agg gat agg ctt acc agt tcc aac ctt cgc cga tgc-3' (SEQ ID NO:24) that includes an EcoRI site (gaattc) and the 14 amino acid epitope for the V5 antibody. The oligonucleotides were used for PCR under standard conditions that included 25 cycles of PCR (95^0C for 1 minute, 55^0C for 1 minute, 72^0C for 1.5 minutes for 25 cycles followed by 3 minutes at 72^0C). The PCR fragment was purified by gel electrophoresis and cloned into pTA2.1 (InVitrogen) by standard cloning methods (Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, THIRD EDITION, 2001), creating the plasmid pTA2.1-hPMS134. The pTA2.1-hPMS134 plasmid was digested with the restriction enzyme EcoRI to release the insert (there are two EcoRI restriction sites in the multiple cloning site of pTA2.1 that flank the insert) and the fragment was end-filled using Klenow fragment and dNTPs. Next, the fragment was gel purified, digested with NdeI, and inserted in pT7-Ea (which had been digested with NdeI and BamHI, end-filledusing Klenow, and phosphatase treated). The new plasmid was designated pT7-Ea-hPMS134.

The following strategy, similar to that described above to clone human PMS134, was used to construct an expression vector for the human related gene PMSR3. First, the hPMSR3 fragment was amplified by PCR to introduce two restriction sites: an NdeI restriction site at the 5'- end, and an Eco RI site at the 3'-end of the fragment. The 5'-oligonucleotide that was used for PCR has the following sequence: 5'-acg cat atg tgt cct tgg cgg cct aga-3' (SEQ ID NO:25) that includes the NdeI restriction site (CAT ATG). The 3'-oligonucleotide used for PCR has the following sequence: 5'-gaa ttc tta tta cgt aga atc gag acc gag gag agg gtt agg gat agg ctt acc cat gtg tga tgt ttc aga gct-3' (SEQ ID NO:26) that includes an EcoRI site (gaattc) and the V5 epitope to allow for antibody detection. The plasmid that contained human PMSR3 in pBluescript SK (Nicolaides *et al.* (1995) *Genomics* 30:195-206) was used as the PCR target with the hPMS2-specific oligonucleotides above. Following 25 cycles of PCR (95^0C for 1 minute, 55^0C for 1 minute, 72^0C for 1.5 minutes for 25 cycles followed by 3

minutes at 72^{0}C). The PCR fragment was purified by gel electrophoresis and cloned into pTA2.1 (InVitrogen) by standard cloning methods (Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, THIRD EDITION, 2001), creating the plasmid pTA2.1-hR3. The pTA2.1-hR3 plasmid was next digested with the restriction enzyme EcoRI to release the insert (there are two EcoRI restriction sites in the multiple cloning site of pTA2.1 that flank the insert) and the fragment was end-filled using Klenow fragment and dNTPs. Then, the fragment was gel purified, digested with NdeI, and inserted in pT7-Ea (which had been digested with NdeI and BamHI, end-filled using Klenow, and phosphatase treated). The new plasmid was designated pT7-Ea-hR3.

BL21 cells harbor an additional expression vector for the lysozyme protein, which has been demonstrated to bind to the T7 polymerase *in situ*; this results in a bacterial strain that has very low levels of T7 polymerase expression. However, upon addition of the inducer isopropyl-beta-D-thiogalactopyranoside (IPTG), the cells express high-levels of T7 polymerase due to the IPTG-inducible element that drives expression of the polymerase that is resident within the genome of the BL21 cells (Studier *et al.* (1991) *J. Mol. Biol.* 219(1):37-44). The BL21 cells are chloramphenicol resistant due to the plasmid that expresses lysozyme within the cell. To introduce the pT7-hPMS134 or the pT7-hPMSR3 genes into BL21 cells, the cells were made competent by incubating the cells in ice cold 50mM $CaCl_2$ for 20 minutes, followed by concentrating the cells and adding super-coiled plasmid DNA as describe (Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, THIRD EDITION, Cold Spring Harbor Laboratory Press, 2001). Ampicillin resistant BL21 were selected on LB-agar plates [5% yeast extract, 10% bactotryptone, 5% NaCl, 1.5% bactoagar, pH 7.0 (Difco)] plates containing 25 μg/ml chloramphenicol and 100μg/ml ampicillin. The next day, bacterial colonies were selected and analyzed by restriction endonuclease digestion and sequence analysis for plasmids containing an intact pTACPMS134 or pTAC empty plasmid .

In addition to constructing a V5-epitope tagged PMS134 construct, we also constructed and tested a non-epitope tagged version. This was prepared to demonstrate that the epitope tag did not cause the alteration of the dominant-negative phenotype that PMS134 has on mismatch repair activity. For these studies, a BamHI restriction fragment containing the hPMS134 cDNA was filled-in using Klenow fragment and then sub-cloned into a

Klenow-filled, blunt-ended NdeI-XhoI site of the pTACLAC expression vector (which contains the IPTG-inducible bacterial TAC promoter and ampicillin resistance gene as selectable marker). The NdeI-XhoI cloning site is flanked by the TACLAC promoter that contains the LacI repressor site followed by a Shine-Dalgarno ribosome-binding site at the 5' flanking region and the T1T2 ribosomal RNA terminator in the 3' flanking region. The TACLAC vector also contains the LacI gene, which is constitutively expressed by the TAC promoter.

DH10B bacterial cells containing the pBCSK vector (Stratagene), which constitutively expresses the β-galactosidase gene and contains the chloramphenicol resistance marker for selection, were made competent via the $CaCl_2$ method (Sambrook, *et al.* MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory Press, 1982). This vector turns bacterial cells blue when grown in the presence of IPTG and X-gal that aids in the detection of bacterial colonies. Competent cells were transfected with the pTAC empty vector or the pTAC*PMS134* vector following the heat-shock protocol. Transfected cultures were plated onto LB-agar [5% yeast extract, 10% bactotryptone, 5% NaCl, 1.5% bactoagar, pH 7.0 (Difco)] plates containing 25 μg/ml chloramphenicol and 100 μg/ml ampicillin. The next day, bacterial colonies were selected and analyzed by restriction endonuclease digestion and sequence analysis for plasmids containing an intact pTACPMS134 or pTAC empty plasmid. Ten clones of each bacteria containing correct empty or PMS134 inserts were then grown to confluence overnight in LB media (5% yeast extract, 10% bactotryptone, 5% NaCl, pH 7.0) containing 10 μg/ml chloramphenicol and 50 μg/ml ampicillin. The next day TAC empty or pTAC*PMS134* cultures were diluted 1:4 in LB medium plus 50 μM IPTG (Gold Biotechnology) and cultures were grown for 12 and 24 hours at 37°C. After incubation, 50 μl aliquots were taken from each culture and added to 150 μls of 2X SDS buffer and cultures were analyzed for PMS134 protein expression by western blot.

Western blots were carried out as follows: 50 μls of each PMS134 or empty plasmid culture was directly lysed in 2X lysis buffer (60 mM Tris, pH 6.8, 2% SDS, 10% glycerol, 0.1 M 2-mercaptoethanol, 0.001% bromophenol blue) and samples were boiled for 5 minutes. Lysate proteins were separated by electrophoresis on 4-20% Tris glycine gels (Novex). Gels were electroblotted onto Immobilon-P (Millipore) in 48 mM Tris base, 40 mM glycine,

0.0375% SDS, 20% methanol and blocked overnight at 4°C in Tris-buffered saline plus 0.05% Tween-20 and 5% condensed milk. Filters were probed with a rabbit polyclonal antibody generated against the N-terminus of the human PMS2 polypeptide (Santa Cruz), which is able to recognize the PMS134 polypeptide (Su *et al.* (1988) *J. Biol. Chem.* 263:6829-6835), followed by a secondary goat anti-rabbit horseradish peroxidase-conjugated antibody. Alternatively, blots were probed with an anti-V5 monoclonal antibody followed by a secondary goat anti-mouse horseradish peroxidase-conjugated antibody. After incubation with the secondary antibody, blots are developed using chemiluminescence (Pierce) and exposed to film to measure PMS134 expression.

For induction of PMS gene product, 5 ml cultures of Luria Broth (LB) plus 50 μg/ml ampicillin were inoculated from glycerol stocks of the transformants pT7Ea (BL21), pT7PMS134/V5 (BL21), or pT7PMSR3 (BL21) and grown overnight at 37°C with shaking. 200 μl of each overnight culture was inoculated in 20 ml (1:100) fresh LB broth plus ampicillin and grown to an OD_{600} of 0.6. 20 μl of 100 mM IPTG (final concentration 0.1mM) was added and cultures were grown overnight. Western analysis confirmed the presence of inducible PMS expression in the presence of inducer molecule (not shown).

Example 2: Generation of antibiotic resistant bacteria

To demonstrate the ability to produce antibiotic resistant bacterial strains by inhibiting MMR, 10^7 bacterial cells expressing either the vector (pT7Ea) or pT7PMS134/V5 were inoculated into 5 ml LB broth plus the appropriate antibiotic concentrations as shown below (Table 1) and grown overnight at 37°C with shaking. Antibiotic concentrations were based on 0.5X the minimum inhibitory concentrations (MIC) observed to inhibit the growth of bacteria constitutively expressing the *mar* operon (Goldman *et al.* (1996) Antimicrobial Agents Chemother. 40: 1266-1269). Titration analysis found the following amounts to be effective in inhibiting bacterial growth in the presence of various compounds.

Table 1. Half minimum inhibitory concentrations (MIC) on BL21 cells.

DRUG	**0.5X MIC (μg/ml)**
Tetracycline	4.70
Nalidixic Acid	7.10
Ofloxacin	0.13
Norfloxacin	0.13
Vancomycin	250.0

The next day, cultures were analyzed for cell growth in the presence or absence of antibiotics. Table 2 summarizes typical data from these studies. No growth was observed in bacterial control cells (pT7Ea), which had OD levels similar to blank culture. In contrast, significant culture growth was observed in pT7PMS134V5 and pT7PMSR3 (not shown) cultures grown in all antibiotics tested (Table 2)

Table 2. Overnight Growth of Drug Resistant Mutants Expressing the PMS2-134.

Drug	**pT7Ea**		**pT7PMS134V5**	
	growth	Cell #	growth	Cell # ($X10^9$)
Tetracycline	-	0	+	1.10
Nalidixic Acid	-	0	+	0.97
Ofloxacin	-	0	+	1.20
Norfloxacin	-	0	+	1.40
Vancomycin	-	0	+	ND

To test the stability of antibiotic resistance, cells were replated and followed for growth in the presence of 1X MIC concentration of antibiotic. Table 3 shows an example in which bacterial cells were inoculated at 1 x 10^7 cells/ml and grown for 6 hours in the presence of tetracycline (Tet). As shown in Figure 1, pT7Ea control culture did not grow in the presence of Tet while pT7PMS134 and pT7PMSR3 cultures resistant to Tet grew to confluence at time 4 hours after inoculation. These data demonstrate the ability to generate

antibiotic resistant cultures by blocking MMR and reestablishing genetically stable cultures that can be used for gene discovery.

EXAMPLE 3: Genomic analysis of antibiotic resistant bacteria and target discovery.

The ability to generate a wide degree of genomic mutation in MMR defective bacteria allow for the rapid analysis of the AR host's genome in comparison to the wild-type strain. While many methods for mutation analysis exist that are know by those skilled in the art, several approaches exist that allow for the screening of unknown genes as well as those that exist which are capable of screening for mutants within "candidate" genes that are capable of conferring an antiobiotic resistant phenotype. One such method includes the use of *in vitro*-coupled-translation strategies, which is a rapid method that is used to screen for mutations that result in truncated polypeptides (Liu *et al.* (1996) *Nat. Med.* 2:169-174; Nicolaides *et al.* (1994) *Nature* 371: 75-80; Papadopoulos *et al.* (1994) *Science* 263:1625-1629; Nicolaides *et al.* (1998) *Mol. Cell. Biol.* 18:1635-1641; Alekshun, M.N. and S.B. Levy (1999) *J. Bacteriol.* 181:3303-3306).

***In vitro* transcription-coupled-translation.**

Linear DNA fragments containing candidate gene sequences were prepared by PCR, incorporating sequences for *in vitro* transcription and translation in the sense primer. The sense primer contains the leader sequence 5'-tttaatacgactcactatagggagaccaccatggnnn nnn nnn nnn nnn-3' (SEQ ID NO:27) where the series of "n" nuclsotides indicates sequence corresponding to the first 5 codons. The antisense primer consists of nucleotide sequences surrounding and including the natural stop codon of the gene. DNA fragments are PCR amplified using buffers and condions as described (Nicolaides *et al.* (1995) *Genomics* 30:195-206). Two to five microliters of whole bacteria are added to the PCR reaction mix and reactions are carried out at 95°C for 1 minute for one cycle followed by thirty cycles at 95°C for 30 sec, 52°C for 2 minute and 72°C for 2 minutes. PCR products are then directly added to a rabbiti reticulolysate mixture to carry out transcription-coupled-translation (Promega). The reaction mixtures were supplemented with [^{35}S]-methionine for detection purposes. Translation reactions are incubated for 2 hours. After the reaction is complete, an equal volume of 2X SDS lysis buffer

is added to the samples, and the samples are boiled and then loaded onto 12% NuPAGE gels (Novex). Gels are run at 150V, dried and exposed to autoradiography. Products that are smaller than the expected molecular weight of the wild-type protein (as compared to the control samples) are then determined to be mutant and DNA fragments are sequenced to confirm the presence of a frame-shift/nonsense mutation. This approach has been used to identify mutations in bacterial genes that have been previously been reported to produce antibiotic resistance in bacteria.

Discussion

The results described above lead to several conclusions. The inhibition of MMR results in an increase in hypermutability in bacteria. This activity is due to the inhibition of MMR biochemical activity in these hosts. This invention provides a novel method of producing antibiotic resistant strains for target discovery and the rational design of novel anti-microbial agents to each target identified by generating AR bacteria through the inhibition of mismatch repair.

The disclosures of the following references, as well as the references cited herein, are hereby incorporated by reference in their entirety.

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What is claimed is:

1. A method for generating antibiotic resistant bacteria comprising the steps of:

blocking mismatch repair in a bacterium whereby said bacterium becomes hypermutable;

contacting said bacterium with at least one antibiotic;

selecting said a bacterium that is resistant to said antibiotic; and

culturing said bacterium;

thereby generating antibiotic resistant bacteria.

2. The method of claim 1 wherein said mismatch repair is blocked by introducing a dominant negative allele of a mismatch repair gene into said bacterium.

3. The method of claim 2 wherein said dominant negative allele of a mismatch repair gene is a *PMS2*-134 gene.

4. The method of claim 1 wherein said mismatch repair is blocked by introducing an antisense nucleic acid molecule into said bacterium wherein said antisense nucleic acid molecule specifically binds to a mismatch repair gene and inhibits mismatch repair in said bacterium.

5. The method of claim 1 wherein said mismatch repair is blocked by exposing said bacterium to a compound that inhibits mismatch repair.

6. The method of claim 5 wherein said compound is an anthracene derivative having the formula:

wherein R_1-R_{10} are independently hydrogen, hydroxyl, amino, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl,O-alkynyl, S-alkynyl, N-alkynyl, aryl, substituted aryl, aryloxy,

substituted aryloxy, heteroaryl, substituted heteroaryl, aralkyloxy, arylalkyl, alkylaryl, alkylaryloxy, arylsulfonyl, alkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl, guanidino, carboxy, an alcohol, an amino acid, sulfonate, alkyl sulfonate, CN, NO_2, an aldehyde group, an ester, an ether, a crown ether, a ketone, an organosulfur compound, an organometallic group, a carboxylic acid, an organosilicon or a carbohydrate that optionally contains one or more alkylated hydroxyl groups;

wherein said heteroalkyl, heteroaryl, and substituted heteroaryl contain at least one heteroatom that is oxygen, sulfur, a metal atom, phosphorus, silicon or nitrogen;

wherein said substituents of said substituted alkyl, substituted alkenyl, substituted alkynyl, substituted aryl, and substituted heteroaryl are halogen, CN, NO_2, lower alkyl, aryl, heteroaryl, aralkyl, aralkyloxy, guanidino, alkoxycarbonyl, alkoxy, hydroxy, carboxy and amino; and

wherein said amino groups optionally substituted with an acyl group, or 1 to 3 aryl or lower alkyl groups.

7. The method of claim 6 wherein said compound is selected from the group consisting of 1,2-dimethylanthracene, 9,10-dimethyl anthracene, 7,8-dimethylanthracene, 9,10-diphenylanthracene, 9,10-dihydroxymethylanthracene, 9-hydroxymethyl-10-methylanthracene, dimethylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-3,4-diol, 9,10-di-m-tolyanthracene.

8. The method of claim 6, further comprising exposing said bacterium to a chemical mutagen.

9. The method of claim 8 wherein said chemical mutagen is selected from the group consisting of methane sulfonate, dimethyl sulfonate, O-6-methyl benzadine, ethylnitrosourea, ethidium bromide, ethyl methanesulfonate, N-methyl-N'-nitro-N-nitrosoguanidine, methylnitrosourea, Tamoxifen, and 8-hydroxyguanine.

10. The method of claim 5 wherein said compound is selected from the group consisting of

an ATP analog, a nuclease inhibitor, and a DNA polymerase inhibitor.

11. The method of claim 10 wherein said ATP analog is selected from the group consisting of AMP-PNP and ATP[gamma]S.

12. The method of claim 10 wherein said nuclease inhibitor is selected from the group consisting of N-ethylmaleimide, heterodimeric adenine-chain-acridine compounds, exonulcease III inhibitors and heliquinomycin.

13. The method of claim 10 wherein said DNA polymerase inhibitor is selected from the group consisting of actinomycin D analogs, aphidicolin, 1-(2'-Deoxy-2'-fluoro-beta-L-arabinofuranosyl)-5-methyluracil, and 2',3'-dideoxyribonucleoside 5'-triphosphates.

14. The method of claim 1 wherein said antibiotic is a quinilone.

15. The method of claim 1 wherein said antibiotic is an aminoglycoside.

16. The method of claim 1 wherein said antibiotic is a magainin.

17. The method of claim 1 wherein said antibiotic is a defensin.

18. The method of claim 1 wherein said antibiotic is a tetracycline.

19. The method of claim 1 wherein said antibiotic is a beta-lactam.

20. The method of claim 1 wherein said antibiotic is a macrolide.

21. The method of claim 1 wherein said antibiotic is a lincosamide.

22. The method of claim 1 wherein said antibiotic is a sulfonamide.

23. The method of claim 1 wherein said antibiotic is a chloramphenicol.

24. The method of claim 1 wherein said antibiotic is a nitrofurantoin.

25. The method of claim 1 wherein said antibiotic is an isoniazid.

26. The method of claim 1 wherein the step of determining whether said bacterium is resistant to said antibiotic comprises analyzing said bacterium for multiantiboitic resistance.

27. The method of claim 1 further comprising making antibiotic resistant bacteria genetically stable.

28. The method of claim 5 further comprising making antibiotic resistant bacteria genetically stable.

29. The method of claim 28 wherein said antibiotic resistant bacteria are made genetically stable by removing the MMR inhibitory molecule.

30. A method for identifying a mutant gene conferring antibiotic resistance comprising comparing the genome of antibiotic resistant bacterium made by the method of claim 1 to the genome of a wild-type strain of said bacterium.

31. The method of claim 30 wherein the genome of said antibiotic resistant bacterium and the genome of said wild-type strain of said bacterium are compared by sequence analysis of the entire genomes.

32. The method of claim 30 wherein the genome of said antibiotic resistant bacterium and the genome of said wild-type strain of said bacterium are compared by microarray analysis.

33. The method of claim 30 wherein the genome of said antibiotic resistant bacterium and the genome of said wild-type strain of said bacterium are compared by:

introducing gene fragments from said antibiotic resistant bacterium into the wild-type bacterium, thereby producing mutant bacteria;

selecting a mutant bacterium with antibiotic resistance; and

sequencing said gene fragment from said mutant bacterium with antibiotic resistance, thereby identifying the antibiotic resistance gene.

34. The method of claim 30 wherein the genome of said antibiotic resistant bacterium and the genome of said wild-type bacterium are compared by:

introducing gene fragments from said wild-type strain of said bacterium into the antibiotic resistant strain of said bacterium;

selecting a mutant bacterium with antibiotic resistance; and

sequencing said gene fragment from said mutant bacterium, thereby identifying the antibiotic resistance gene.

35. A method of producing an antibiotic resistant bacterium comprising the steps of:

culturing bacteria with a natural defect in mismatch repair;

contacting said bacteria with at least one antibiotic;

selecting a bacterium among said bacteria resistant to said antibiotic; and

culturing said bacterium;

thereby generating antibiotic resistant bacteria.

36. A method of generating antibiotic resistant bacteria comprising the steps of:

overexpressing a mismatch repair gene in a bacterium whereby said bacterium becomes hypermutable;

contacting said bacterium with at least one antibiotic;

determining whether said bacterium is resistant to said antibiotic; and

culturing said bacterium;

thereby generating antibiotic resistant bacteria.

37. The method of claim 36 further comprising making said antibiotic resistant bacteria genetically stable.

38. An antibiotic resistant bacterium produced by the method of claim 1.

39. An antibiotic resistant bacterium produced by the method of claim 35.

40. An antibiotic resistant bacterium produced by the method of claim 36.

41. An antibiotic resistant bacterium produced by the method of claim 37.

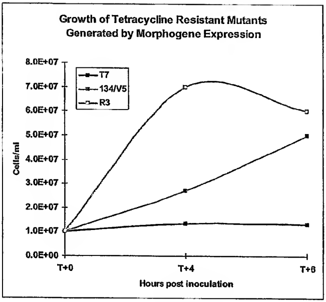
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Figure 1

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Phe Asp Lys Ile Glu Val Arg Asp Asn Gly Glu Gly Ile Lys Ala Val
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Asp Ala Pro Val Met Ala Met Lys Tyr Tyr Thr Ser Lys Ile Asn Ser
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His Glu Asp Leu Glu Asn Leu Thr Thr Tyr Gly Phe Arg Gly Glu Ala
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Leu Gly Ser Ile Cys Cys Ile Ala Glu Val Leu Ile Thr Thr Arg Thr
100 105 110

Ala Ala Asp Asn Phe Ser Thr Gln Tyr Val Leu Asp Gly Ser Gly His
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Ile Leu Ser Gln Lys Pro Ser His Leu Gly Gln Gly Thr Thr Val Thr
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Ala Leu Arg Leu Phe Lys Asn Leu Pro Val Arg Lys Gln Phe Tyr Ser
145 150 155 160

Thr Ala Lys Lys Cys Lys Asp Glu Ile Lys Lys Ile Gln Asp Leu Leu
165 170 175

Met Ser Phe Gly Ile Leu Lys Pro Asp Leu Arg Ile Val Phe Val His
180 185 190

Asn Lys Ala Val Ile Trp Gln Lys Ser Arg Val Ser Asp His Lys Met
195 200 205

Ala Leu Met Ser Val Leu Gly Thr Ala Val Met Asn Asn Met Glu Ser
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Phe Gln Tyr His Ser Glu Glu Ser Gln Ile Tyr Leu Ser Gly Phe Leu
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Pro Lys Cys Asp Ala Asp His Ser Phe Thr Ser Leu Ser Thr Pro Glu
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Arg Ser Phe Ile Phe Ile Asn Ser Arg Pro Val His Gln Lys Asp Ile
260 265 270

Leu Lys Leu Ile Arg His His Tyr Asn Leu Lys Cys Leu Lys Glu Ser
275 280 285

Thr Arg Leu Tyr Pro Val Phe Phe Leu Lys Ile Asp Val Pro Thr Ala
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Asp Val Asp Val Asn Leu Thr Pro Asp Lys Ser Gln Val Leu Leu Gln
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Asn Lys Glu Ser Val Leu Ile Ala Leu Glu Asn Leu Met Thr Thr Cys
325 330 335

Tyr Gly Pro Leu Pro Ser Thr Asn Ser Tyr Glu Asn Asn Lys Thr Asp
340 345 350

Val Ser Ala Ala Asp Ile Val Leu Ser Lys Thr Ala Glu Thr Asp Val
355 360 365

Leu Phe Asn Lys Val Glu Ser Ser Gly Lys Asn Tyr Ser Asn Val Asp
370 375 380

Thr Ser Val Ile Pro Phe Gln Asn Asp Met His Asn Asp Glu Ser Gly
385 390 395 400

Lys Asn Thr Asp Asp Cys Leu Asn His Gln Ile Ser Ile Gly Asp Phe
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Gly Tyr Gly His Cys Ser Ser Glu Ile Ser Asn Ile Asp Lys Asn Thr
420 425 430

Lys Asn Ala Phe Gln Asp Ile Ser Met Ser Asn Val Ser Trp Glu Asn
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Ser Gln Thr Glu Tyr Ser Lys Thr Cys Phe Ile Ser Ser Val Lys His
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Thr Gln Ser Glu Asn Gly Asn Lys Asp His Ile Asp Glu Ser Gly Glu
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Asn Glu Glu Glu Ala Gly Leu Glu Asn Ser Ser Glu Ile Ser Ala Asp
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Trp Lys Thr Leu Ser Glu Glu Glu Lys Leu Lys Tyr Glu Glu Lys Ala
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Thr Lys Asp Leu Glu Arg Tyr Asn Ser Gln Met Lys Arg Ala Ile Glu
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Gln Glu Ser Gln Met Ser Leu Lys Asp Gly Arg Lys Lys Ile Lys Pro
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Ser Asn Gln Pro Lys Leu Asp Glu Leu Leu Gln Ser Gln Ile Glu Lys
675 680 685

Arg Arg Ser Gln Asn Ile Lys Met Val Gln Ile Pro Phe Ser Met Lys
690 695 700

Asn Leu Lys Ile Asn Phe Lys Lys Gln Asn Lys Val Asp Leu Glu Glu
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Lys Asp Glu Pro Cys Leu Ile His Asn Leu Arg Phe Pro Asp Ala Trp
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Leu Met Thr Ser Lys Thr Glu Val Met Leu Leu Asn Pro Tyr Arg Val
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Glu Glu Ala Leu Leu Phe Lys Arg Leu Leu Glu Asn His Lys Leu Pro
755 760 765

Ala Glu Pro Leu Glu Lys Pro Ile Met Leu Thr Glu Ser Leu Phe Asn
770 775 780

Gly Ser His Tyr Leu Asp Val Leu Tyr Lys Met Thr Ala Asp Asp Gln
785 790 795 800

Arg Tyr Ser Gly Ser Thr Tyr Leu Ser Asp Pro Arg Leu Thr Ala Asn
805 810 815

Gly Phe Lys Ile Lys Leu Ile Pro Gly Val Ser Ile Thr Glu Asn Tyr
820 825 830

Leu Glu Ile Glu Gly Met Ala Asn Cys Leu Pro Phe Tyr Gly Val Ala
835 840 845

Asp Leu Lys Glu Ile Leu Asn Ala Ile Leu Asn Arg Asn Ala Lys Glu
850 855 860

Val Tyr Glu Cys Arg Pro Arg Lys Val Ile Ser Tyr Leu Glu Gly Glu
865 870 875 880

Ala Val Arg Leu Ser Arg Gln Leu Pro Met Tyr Leu Ser Lys Glu Asp
885 890 895

Ile Gln Asp Ile Ile Tyr Arg Met Lys His Gln Phe Gly Asn Glu Ile
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Pro Glu Thr Thr
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Leu Asp Ala Gly Ala Thr Ser Val Asp Val Lys Leu Glu Asn Tyr Gly
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Phe Asp Lys Ile Glu Val Arg Asp Asn Gly Glu Gly Ile Lys Ala Val
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Asp Ala Pro Val Met Ala Met Lys Tyr Tyr Thr Ser Lys Ile Asn Ser
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His Glu Asp Leu Glu Asn Leu Thr Thr Tyr Gly Phe Arg Gly Glu Ala
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Leu Gly Ser Ile Cys Cys Ile Ala Glu Val Leu Ile Thr Thr Arg Thr
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Ala Ala Asp Asn Phe Ser Thr Gln Tyr Val Leu Asp Gly Ser Gly His
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Ile Leu Ser Gln Lys Pro Ser His Leu Gly Gln Gly Thr Thr Val Thr
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Ala Leu Arg Leu Phe Lys Asn Leu Pro Val Arg Lys Gln Phe Tyr Ser
145 150 155 160

Thr Ala Lys Lys Cys Lys Asp Glu Ile Lys Lys Ile Gln Asp Leu Leu
165 170 175

Met Ser Phe Gly Ile Leu Lys Pro Asp Leu Arg Ile Val Phe Val His
180 185 190

Asn Lys Ala Val Ile Trp Gln Lys Ser Arg Val Ser Asp His Lys Met
195 200 205

Ala Leu Met Ser Val Leu Gly Thr Ala Val Met Asn Asn Met Glu Ser
210 215 220

Phe Gln Tyr His Ser Glu Glu Ser Gln Ile Tyr Leu Ser Gly Phe Leu
225 230 235 240

Pro Lys Cys Asp Ala Asp His Ser Phe Thr Ser Leu Ser Thr Pro Glu
245 250 255

Arg Ser Phe Ile Phe Ile Asn Ser Arg Pro Val His Gln Lys Asp Ile
260 265 270

Leu Lys Leu Ile Arg His His Tyr Asn Leu Lys Cys Leu Lys Glu Ser
275 280 285

Thr Arg Leu Tyr Pro Val Phe Phe Leu Lys Ile Asp Val Pro Thr Ala
290 295 300

Asp Val Asp Val Asn Leu Thr Pro Asp Lys Ser Gln Val Leu Leu Gln
305 310 315 320

Asn Lys Glu Ser Val Leu Ile Ala Leu Glu Asn Leu Met Thr Thr Cys
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Tyr Gly Pro Leu Pro Ser Thr Asn Ser Tyr Glu Asn Asn Lys Thr Asp

340 345 350

Val Ser Ala Ala Asp Ile Val Leu Ser Lys Thr Ala Glu Thr Asp Val
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Leu Phe Asn Lys Val Glu Ser Ser Gly Lys Asn Tyr Ser Asn Val Asp
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Thr Ser Val Ile Pro Phe Gln Asn Asp Met His Asn Asp Glu Ser Gly
385 390 395 400

Lys Asn Thr Asp Asp Cys Leu Asn His Gln Ile Ser Ile Gly Asp Phe
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Gly Tyr Gly His Cys Ser Ser Glu Ile Ser Asn Ile Asp Lys Asn Thr
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Lys Asn Ala Phe Gln Asp Ile Ser Met Ser Asn Val Ser Trp Glu Asn
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Ser Gln Thr Glu Tyr Ser Lys Thr Cys Phe Ile Ser Ser Val Lys His
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Asn Glu Glu Glu Ala Gly Leu Glu Asn Ser Ser Glu Ile Ser Ala Asp
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Glu Trp Ser Arg Gly Asn Ile Leu Lys Asn Ser Val Gly Glu Asn Ile
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Glu Pro Val Lys Ile Leu Val Pro Glu Lys Ser Leu Pro Cys Lys Val
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Ser Cys Asn Lys Lys Ser Asn Val Ile Asp Asn Lys Ser Gly Lys Val
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Ala Ser Ala Leu Phe Val Gln Asp His Arg Pro Gln Phe Leu Ile Glu
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Asn Pro Lys Thr Ser Leu Glu Asp Ala Thr Leu Gln Ile Glu Glu Leu

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Trp Lys Thr Leu Ser Glu Glu Glu Lys Leu Lys Tyr Glu Glu Lys Ala
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Thr Lys Asp Leu Glu Arg Tyr Asn Ser Gln Met Lys Arg Ala Ile Glu
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Gln Glu Ser Gln Met Ser Leu Lys Asp Gly Arg Lys Lys Ile Lys Pro
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Thr Ser Ala Trp Asn Leu Ala Gln Lys His Lys Leu Lys Thr Ser Leu
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Ser Asn Gln Pro Lys Leu Asp Glu Leu Leu Gln Ser Gln Ile Glu Lys
675 680 685

Arg Arg Ser Gln Asn Ile Lys Met Val Gln Ile Pro Phe Ser Met Lys
690 695 700

Asn Leu Lys Ile Asn Phe Lys Lys Gln Asn Lys Val Asp Leu Glu Glu
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Lys Asp Glu Pro Cys Leu Ile His Asn Leu Arg Phe Pro Asp Ala Trp
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Leu Met Thr Ser Lys Thr Glu Val Met Leu Leu Asn Pro Tyr Arg Val
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Glu Glu Ala Leu Leu Phe Lys Arg Leu Leu Glu Asn His Lys Leu Pro
755 760 765

Ala Glu Pro Leu Glu Lys Pro Ile Met Leu Thr Glu Ser Leu Phe Asn
770 775 780

Gly Ser His Tyr Leu Asp Val Leu Tyr Lys Met Thr Ala Asp Asp Gln
785 790 795 800

Arg Tyr Ser Gly Ser Thr Tyr Leu Ser Asp Pro Arg Leu Thr Ala Asn
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Gly Phe Lys Ile Lys Leu Ile Pro Gly Val Ser Ile Thr Glu Asn Tyr
820 825 830

Leu Glu Ile Glu Gly Met Ala Asn Cys Leu Pro Phe Tyr Gly Val Ala
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Asp Leu Lys Glu Ile Leu Asn Ala Ile Leu Asn Arg Asn Ala Lys Glu

850 855 860

Val Tyr Glu Cys Arg Pro Arg Lys Val Ile Ser Tyr Leu Glu Gly Glu
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Ala Val Arg Leu Ser Arg Gln Leu Pro Met Tyr Leu Ser Lys Glu Asp
885 890 895

Ile Gln Asp Ile Ile Tyr Arg Met Lys His Gln Phe Gly Asn Glu Ile
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Lys Glu Cys Val His Gly Arg Pro Phe Phe His His Leu Thr Tyr Leu
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Asp Ala Leu Leu Ala Ala Arg Glu Val Phe Lys Thr Gln Gly Val Ile
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Lys Tyr Met Gly Pro Ala Gly Ala Lys Asn Leu Gln Ser Val Val Leu
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Ser Lys Met Asn Phe Glu Ser Phe Val Lys Asp Leu Leu Leu Val Arg
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Gln Tyr Arg Val Glu Val Tyr Lys Asn Arg Ala Gly Asn Lys Ala Ser
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Lys Glu Asn Asp Trp Tyr Leu Ala Tyr Lys Ala Ser Pro Gly Asn Leu
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Ser Gln Phe Glu Asp Ile Leu Phe Gly Asn Asn Asp Met Ser Ala Ser
130 135 140

Ile Gly Val Val Gly Val Lys Met Ser Ala Val Asp Gly Gln Arg Gln
145 150 155 160

Val Gly Val Gly Tyr Val Asp Ser Ile Gln Arg Lys Leu Gly Leu Cys
165 170 175

Glu Phe Pro Asp Asn Asp Gln Phe Ser Asn Leu Glu Ala Leu Leu Ile
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Gln Ile Gly Pro Lys Glu Cys Val Leu Pro Gly Gly Glu Thr Ala Gly

195 200 205

Asp Met Gly Lys Leu Arg Gln Ile Ile Gln Arg Gly Gly Ile Leu Ile
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Thr Glu Arg Lys Lys Ala Asp Phe Ser Thr Lys Asp Ile Tyr Gln Asp
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275 280 285

Phe Glu Leu Thr Thr Phe Asp Phe Ser Gln Tyr Met Lys Leu Asp Ile
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Thr Gly Ser Gln Ser Leu Ala Ala Leu Leu Asn Lys Cys Lys Thr Pro
325 330 335

Gln Gly Gln Arg Leu Val Asn Gln Trp Ile Lys Gln Pro Leu Met Asp
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Lys Asn Arg Ile Glu Glu Arg Leu Asn Leu Val Glu Ala Phe Val Glu
355 360 365

Asp Ala Glu Leu Arg Gln Thr Leu Gln Glu Asp Leu Leu Arg Arg Phe
370 375 380

Pro Asp Leu Asn Arg Leu Ala Lys Lys Phe Gln Arg Gln Ala Ala Asn
385 390 395 400

Leu Gln Asp Cys Tyr Arg Leu Tyr Gln Gly Ile Asn Gln Leu Pro Asn
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Val Ile Gln Ala Leu Glu Lys His Glu Gly Lys His Gln Lys Leu Leu
420 425 430

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Lys Phe Gln Glu Met Ile Glu Thr Thr Leu Asp Met Asp Gln Val Glu

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Asn His Glu Phe Leu Val Lys Pro Ser Phe Asp Pro Asn Leu Ser Glu
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Glu Glu Tyr Thr Lys Asn Lys Thr Glu Tyr Glu Glu Ala Gln Asp Ala
565 570 575

Ile Val Lys Glu Ile Val Asn Ile Ser Ser Gly Tyr Val Glu Pro Met
580 585 590

Gln Thr Leu Asn Asp Val Leu Ala Gln Leu Asp Ala Val Val Ser Phe
595 600 605

Ala His Val Ser Asn Gly Ala Pro Val Pro Tyr Val Arg Pro Ala Ile
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Cys Val Glu Val Gln Asp Glu Ile Ala Phe Ile Pro Asn Asp Val Tyr
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Phe Glu Lys Asp Lys Gln Met Phe His Ile Ile Thr Gly Pro Asn Met
660 665 670

Gly Gly Lys Ser Thr Tyr Ile Arg Gln Thr Gly Val Ile Val Leu Met
675 680 685

Ala Gln Ile Gly Cys Phe Val Pro Cys Glu Ser Ala Glu Val Ser Ile
690 695 700

Val Asp Cys Ile Leu Ala Arg Val Gly Ala Gly Asp Ser Gln Leu Lys

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Gly Val Ser Thr Phe Met Ala Glu Met Leu Glu Thr Ala Ser Ile Leu
725 730 735

Arg Ser Ala Thr Lys Asp Ser Leu Ile Ile Ile Asp Glu Leu Gly Arg
740 745 750

Gly Thr Ser Thr Tyr Asp Gly Phe Gly Leu Ala Trp Ala Ile Ser Glu
755 760 765

Tyr Ile Ala Thr Lys Ile Gly Ala Phe Cys Met Phe Ala Thr His Phe
770 775 780

His Glu Leu Thr Ala Leu Ala Asn Gln Ile Pro Thr Val Asn Asn Leu
785 790 795 800

His Val Thr Ala Leu Thr Thr Glu Glu Thr Leu Thr Met Leu Tyr Gln
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Val Lys Lys Gly Val Cys Asp Gln Ser Phe Gly Ile His Val Ala Glu
820 825 830

Leu Ala Asn Phe Pro Lys His Val Ile Glu Cys Ala Lys Gln Lys Ala
835 840 845

Leu Glu Leu Glu Glu Phe Gln Tyr Ile Gly Glu Ser Gln Gly Tyr Asp
850 855 860

Ile Met Glu Pro Ala Ala Lys Lys Cys Tyr Leu Glu Arg Glu Gln Gly
865 870 875 880

Glu Lys Ile Ile Gln Glu Phe Leu Ser Lys Val Lys Gln Met Pro Phe
885 890 895

Thr Glu Met Ser Glu Glu Asn Ile Thr Ile Lys Leu Lys Gln Leu Lys
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Arg Ile Lys Val Thr Thr
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35 40 45

Val Ile Val Lys Glu Gly Gly Leu Lys Leu Ile Gln Ile Gln Asp Asn
50 55 60

Gly Thr Gly Ile Arg Lys Glu Asp Leu Asp Ile Val Cys Glu Arg Phe
65 70 75 80

Thr Thr Ser Lys Leu Gln Ser Phe Glu Asp Leu Ala Ser Ile Ser Thr
85 90 95

Tyr Gly Phe Arg Gly Glu Ala Leu Ala Ser Ile Ser His Val Ala His
100 105 110

Val Thr Ile Thr Thr Lys Thr Ala Asp Gly Lys Cys Ala Tyr Arg Ala
115 120 125

Ser Tyr Ser Asp Gly Lys Leu Lys Ala Pro Pro Lys Pro Cys Ala Gly
130 135 140

Asn Gln Gly Thr Gln Ile Thr Val Glu Asp Leu Phe Tyr Asn Ile Ala
145 150 155 160

Thr Arg Arg Lys Ala Leu Lys Asn Pro Ser Glu Glu Tyr Gly Lys Ile
165 170 175

Leu Glu Val Val Gly Arg Tyr Ser Val His Asn Ala Gly Ile Ser Phe
180 185 190

Ser Val Lys Lys Gln Gly Glu Thr Val Ala Asp Val Arg Thr Leu Pro
195 200 205

Asn Ala Ser Thr Val Asp Asn Ile Arg Ser Ile Phe Gly Asn Ala Val
210 215 220

Ser Arg Glu Leu Ile Glu Ile Gly Cys Glu Asp Lys Thr Leu Ala Phe
225 230 235 240

Lys Met Asn Gly Tyr Ile Ser Asn Ala Asn Tyr Ser Val Lys Lys Cys
245 250 255

Ile Phe Leu Leu Phe Ile Asn His Arg Leu Val Glu Ser Thr Ser Leu
260 265 270

Arg Lys Ala Ile Glu Thr Val Tyr Ala Ala Tyr Leu Pro Lys Asn Thr
275 280 285

His Pro Phe Leu Tyr Leu Ser Leu Glu Ile Ser Pro Gln Asn Val Asp
290 295 300

Val Asn Val His Pro Thr Lys His Glu Val His Phe Leu His Glu Glu
305 310 315 320

Ser Ile Leu Glu Arg Val Gln Gln His Ile Glu Ser Lys Leu Leu Gly
325 330 335

Ser Asn Ser Ser Arg Met Tyr Phe Thr Gln Thr Leu Leu Pro Gly Leu
340 345 350

Ala Gly Pro Ser Gly Glu Met Val Lys Ser Thr Thr Ser Leu Thr Ser
355 360 365

Ser Ser Thr Ser Gly Ser Ser Asp Lys Val Tyr Ala His Gln Met Val
370 375 380

Arg Thr Asp Ser Arg Glu Gln Lys Leu Asp Ala Phe Leu Gln Pro Leu
385 390 395 400

Ser Lys Pro Leu Ser Ser Gln Pro Gln Ala Ile Val Thr Glu Asp Lys
405 410 415

Thr Asp Ile Ser Ser Gly Arg Ala Arg Gln Gln Asp Glu Glu Met Leu
420 425 430

Glu Leu Pro Ala Pro Ala Glu Val Ala Ala Lys Asn Gln Ser Leu Glu
435 440 445

Gly Asp Thr Thr Lys Gly Thr Ser Glu Met Ser Glu Lys Arg Gly Pro
450 455 460

Thr Ser Ser Asn Pro Arg Lys Arg His Arg Glu Asp Ser Asp Val Glu
465 470 475 480

Met Val Glu Asp Asp Ser Arg Lys Glu Met Thr Ala Ala Cys Thr Pro
485 490 495

Arg Arg Arg Ile Ile Asn Leu Thr Ser Val Leu Ser Leu Gln Glu Glu
500 505 510

Ile Asn Glu Gln Gly His Glu Val Leu Arg Glu Met Leu His Asn His
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Ser Phe Val Gly Cys Val Asn Pro Gln Trp Ala Leu Ala Gln His Gln
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Thr Lys Leu Tyr Leu Leu Asn Thr Thr Lys Leu Ser Glu Glu Leu Phe
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Tyr Gln Ile Leu Ile Tyr Asp Phe Ala Asn Phe Gly Val Leu Arg Leu
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Ser Glu Pro Ala Pro Leu Phe Asp Leu Ala Met Leu Ala Leu Asp Ser
580 585 590

Pro Glu Ser Gly Trp Thr Glu Glu Asp Gly Pro Lys Glu Gly Leu Ala
595 600 605

Glu Tyr Ile Val Glu Phe Leu Lys Lys Lys Ala Glu Met Leu Ala Asp
610 615 620

Tyr Phe Ser Leu Glu Ile Asp Glu Glu Gly Asn Leu Ile Gly Leu Pro
625 630 635 640

Leu Leu Ile Asp Asn Tyr Val Pro Pro Leu Glu Gly Leu Pro Ile Phe
645 650 655

Ile Leu Arg Leu Ala Thr Glu Val Asn Trp Asp Glu Glu Lys Glu Cys
660 665 670

Phe Glu Ser Leu Ser Lys Glu Cys Ala Met Phe Tyr Ser Ile Arg Lys
675 680 685

Gln Tyr Ile Ser Glu Glu Ser Thr Leu Ser Gly Gln Gln Ser Glu Val
690 695 700

Pro Gly Ser Ile Pro Asn Ser Trp Lys Trp Thr Val Glu His Ile Val
705 710 715 720

Tyr Lys Ala Leu Arg Ser His Ile Leu Pro Pro Lys His Phe Thr Glu
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Phe Glu Arg Cys
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Phe Asp Lys Ile Glu Val Arg Asp Asn Gly Glu Gly Ile Lys Ala Val
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Asp Ala Pro Val Met Ala Met Lys Tyr Tyr Thr Ser Lys Ile Asn Ser
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His Glu Asp Leu Glu Asn Leu Thr Thr Tyr Gly Phe Arg Gly Glu Ala
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Leu Gly Ser Ile Cys Cys Ile Ala Glu Val Leu Ile Thr Thr Arg Thr
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gtggatgaat taggaagagg tactgcaaca tttgatggga cggcaatagc aaatgcagtt 3780
gttaaagaac ttgctgagac tataaaatgt cgtacattat tttcaactca ctaccattca 3840
ttagtagaag attattctca aaatgttgct gtgcgcctag gacatatggc atgcatggta 3900
gaaaatgaat gtgaagaccc cagccaggag actattacgt tcctctataa attcattaag 3960
ggagcttgtc ctaaaagcta tggctttaat gcagcaaggc ttgctaatct cccagaggaa 4020
gttattcaaa agggacatag aaaagcaaga gaatttgaga agatgaatca gtcactacga 4080
ttatttcggg aagtttgcct ggctagtgaa aggtcaactg tagatgctga agctgtccat 4140
aaattgctga ctttgattaa ggaattatag actgactaca ttggaagctt tgagttgact 4200
tctgaccaaa ggtggtaaat tcagacaaca ttatgatcta ataaacttta ttttttaaaa 4260
atga 4264

<210> 16
<211> 1360
<212> PRT
<213> Homo sapiens

<400> 16

Met Ser Arg Gln Ser Thr Leu Tyr Ser Phe Phe Pro Lys Ser Pro Ala
1 5 10 15

Leu Ser Asp Ala Asn Lys Ala Ser Ala Arg Ala Ser Arg Glu Gly Gly
20 25 30

Arg Ala Ala Ala Ala Pro Gly Ala Ser Pro Ser Pro Gly Gly Asp Ala
35 40 45

Ala Trp Ser Glu Ala Gly Pro Gly Pro Arg Pro Leu Ala Arg Ser Ala
50 55 60

Ser Pro Pro Lys Ala Lys Asn Leu Asn Gly Gly Leu Arg Arg Ser Val
65 70 75 80

Ala Pro Ala Ala Pro Thr Ser Cys Asp Phe Ser Pro Gly Asp Leu Val
85 90 95

Trp Ala Lys Met Glu Gly Tyr Pro Trp Trp Pro Cys Leu Val Tyr Asn
100 105 110

His Pro Phe Asp Gly Thr Phe Ile Arg Glu Lys Gly Lys Ser Val Arg
115 120 125

Val His Val Gln Phe Phe Asp Asp Ser Pro Thr Arg Gly Trp Val Ser
130 135 140

Lys Arg Leu Leu Lys Pro Tyr Thr Gly Ser Lys Ser Lys Glu Ala Gln
145 150 155 160

Lys Gly Gly His Phe Tyr Ser Ala Lys Pro Glu Ile Leu Arg Ala Met
165 170 175

Gln Arg Ala Asp Glu Ala Leu Asn Lys Asp Lys Ile Lys Arg Leu Glu
180 185 190

Leu Ala Val Cys Asp Glu Pro Ser Glu Pro Glu Glu Glu Glu Glu Met
195 200 205

Glu Val Gly Thr Thr Tyr Val Thr Asp Lys Ser Glu Glu Asp Asn Glu
210 215 220

Ile Glu Ser Glu Glu Glu Val Gln Pro Lys Thr Gln Gly Ser Arg Arg
225 230 235 240

Ser Ser Arg Gln Ile Lys Lys Arg Arg Val Ile Ser Asp Ser Glu Ser
245 250 255

Asp Ile Gly Gly Ser Asp Val Glu Phe Lys Pro Asp Thr Lys Glu Glu
260 265 270

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Gly Ser Ser Asp Glu Ile Ser Ser Gly Val Gly Asp Ser Glu Ser Glu
        275                 280                 285

Gly Leu Asn Ser Pro Val Lys Val Ala Arg Lys Arg Lys Arg Met Val
    290                 295                 300

Thr Gly Asn Gly Ser Leu Lys Arg Lys Ser Ser Arg Lys Glu Thr Pro
305                 310                 315                 320

Ser Ala Thr Lys Gln Ala Thr Ser Ile Ser Ser Glu Thr Lys Asn Thr
                325                 330                 335

Leu Arg Ala Phe Ser Ala Pro Gln Asn Ser Glu Ser Gln Ala His Val
            340                 345                 350

Ser Gly Gly Gly Asp Asp Ser Ser Arg Pro Thr Val Trp Tyr His Glu
        355                 360                 365

Thr Leu Glu Trp Leu Lys Glu Glu Lys Arg Arg Asp Glu His Arg Arg
    370                 375                 380

Arg Pro Asp His Pro Asp Phe Asp Ala Ser Thr Leu Tyr Val Pro Glu
385                 390                 395                 400

Asp Phe Leu Asn Ser Cys Thr Pro Gly Met Arg Lys Trp Trp Gln Ile
                405                 410                 415

Lys Ser Gln Asn Phe Asp Leu Val Ile Cys Tyr Lys Val Gly Lys Phe
            420                 425                 430

Tyr Glu Leu Tyr His Met Asp Ala Leu Ile Gly Val Ser Glu Leu Gly
        435                 440                 445

Leu Val Phe Met Lys Gly Asn Trp Ala His Ser Gly Phe Pro Glu Ile
    450                 455                 460

Ala Phe Gly Arg Tyr Ser Asp Ser Leu Val Gln Lys Gly Tyr Lys Val
465                 470                 475                 480

Ala Arg Val Glu Gln Thr Glu Thr Pro Glu Met Met Glu Ala Arg Cys
                485                 490                 495

Arg Lys Met Ala His Ile Ser Lys Tyr Asp Arg Val Val Arg Arg Glu
            500                 505                 510

Ile Cys Arg Ile Ile Thr Lys Gly Thr Gln Thr Tyr Ser Val Leu Glu
        515                 520                 525
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Gly Asp Pro Ser Glu Asn Tyr Ser Lys Tyr Leu Leu Ser Leu Lys Glu
530 535 540

Lys Glu Glu Asp Ser Ser Gly His Thr Arg Ala Tyr Gly Val Cys Phe
545 550 555 560

Val Asp Thr Ser Leu Gly Lys Phe Phe Ile Gly Gln Phe Ser Asp Asp
565 570 575

Arg His Cys Ser Arg Phe Arg Thr Leu Val Ala His Tyr Pro Pro Val
580 585 590

Gln Val Leu Phe Glu Lys Gly Asn Leu Ser Lys Glu Thr Lys Thr Ile
595 600 605

Leu Lys Ser Ser Leu Ser Cys Ser Leu Gln Glu Gly Leu Ile Pro Gly
610 615 620

Ser Gln Phe Trp Asp Ala Ser Lys Thr Leu Arg Thr Leu Leu Glu Glu
625 630 635 640

Glu Tyr Phe Arg Glu Lys Leu Ser Asp Gly Ile Gly Val Met Leu Pro
645 650 655

Gln Val Leu Lys Gly Met Thr Ser Glu Ser Asp Ser Ile Gly Leu Thr
660 665 670

Pro Gly Glu Lys Ser Glu Leu Ala Leu Ser Ala Leu Gly Gly Cys Val
675 680 685

Phe Tyr Leu Lys Lys Cys Leu Ile Asp Gln Glu Leu Leu Ser Met Ala
690 695 700

Asn Phe Glu Glu Tyr Ile Pro Leu Asp Ser Asp Thr Val Ser Thr Thr
705 710 715 720

Arg Ser Gly Ala Ile Phe Thr Lys Ala Tyr Gln Arg Met Val Leu Asp
725 730 735

Ala Val Thr Leu Asn Asn Leu Glu Ile Phe Leu Asn Gly Thr Asn Gly
740 745 750

Ser Thr Glu Gly Thr Leu Leu Glu Arg Val Asp Thr Cys His Thr Pro
755 760 765

Phe Gly Lys Arg Leu Leu Lys Gln Trp Leu Cys Ala Pro Leu Cys Asn
770 775 780

His Tyr Ala Ile Asn Asp Arg Leu Asp Ala Ile Glu Asp Leu Met Val
785 790 795 800

Val Pro Asp Lys Ile Ser Glu Val Val Glu Leu Leu Lys Lys Leu Pro
805 810 815

Asp Leu Glu Arg Leu Leu Ser Lys Ile His Asn Val Gly Ser Pro Leu
820 825 830

Lys Ser Gln Asn His Pro Asp Ser Arg Ala Ile Met Tyr Glu Glu Thr
835 840 845

Thr Tyr Ser Lys Lys Lys Ile Ile Asp Phe Leu Ser Ala Leu Glu Gly
850 855 860

Phe Lys Val Met Cys Lys Ile Ile Gly Ile Met Glu Glu Val Ala Asp
865 870 875 880

Gly Phe Lys Ser Lys Ile Leu Lys Gln Val Ile Ser Leu Gln Thr Lys
885 890 895

Asn Pro Glu Gly Arg Phe Pro Asp Leu Thr Val Glu Leu Asn Arg Trp
900 905 910

Asp Thr Ala Phe Asp His Glu Lys Ala Arg Lys Thr Gly Leu Ile Thr
915 920 925

Pro Lys Ala Gly Phe Asp Ser Asp Tyr Asp Gln Ala Leu Ala Asp Ile
930 935 940

Arg Glu Asn Glu Gln Ser Leu Leu Glu Tyr Leu Glu Lys Gln Arg Asn
945 950 955 960

Arg Ile Gly Cys Arg Thr Ile Val Tyr Trp Gly Ile Gly Arg Asn Arg
965 970 975

Tyr Gln Leu Glu Ile Pro Glu Asn Phe Thr Thr Arg Asn Leu Pro Glu
980 985 990

Glu Tyr Glu Leu Lys Ser Thr Lys Lys Gly Cys Lys Arg Tyr Trp Thr
995 1000 1005

Lys Thr Ile Glu Lys Lys Leu Ala Asn Leu Ile Asn Ala Glu Glu
1010 1015 1020

Arg Arg Asp Val Ser Leu Lys Asp Cys Met Arg Arg Leu Phe Tyr
1025 1030 1035

Asn Phe Asp Lys Asn Tyr Lys Asp Trp Gln Ser Ala Val Glu Cys
1040 1045 1050

Ile Ala Val Leu Asp Val Leu Leu Cys Leu Ala Asn Tyr Ser Arg
1055 1060 1065

Gly Gly Asp Gly Pro Met Cys Arg Pro Val Ile Leu Leu Pro Glu
1070 1075 1080

Asp Thr Pro Pro Phe Leu Glu Leu Lys Gly Ser Arg His Pro Cys
1085 1090 1095

Ile Thr Lys Thr Phe Phe Gly Asp Asp Phe Ile Pro Asn Asp Ile
1100 1105 1110

Leu Ile Gly Cys Glu Glu Glu Glu Gln Glu Asn Gly Lys Ala Tyr
1115 1120 1125

Cys Val Leu Val Thr Gly Pro Asn Met Gly Gly Lys Ser Thr Leu
1130 1135 1140

Met Arg Gln Ala Gly Leu Leu Ala Val Met Ala Gln Met Gly Cys
1145 1150 1155

Tyr Val Pro Ala Glu Val Cys Arg Leu Thr Pro Ile Asp Arg Val
1160 1165 1170

Phe Thr Arg Leu Gly Ala Ser Asp Arg Ile Met Ser Gly Glu Ser
1175 1180 1185

Thr Phe Phe Val Glu Leu Ser Glu Thr Ala Ser Ile Leu Met His
1190 1195 1200

Ala Thr Ala His Ser Leu Val Leu Val Asp Glu Leu Gly Arg Gly
1205 1210 1215

Thr Ala Thr Phe Asp Gly Thr Ala Ile Ala Asn Ala Val Val Lys
1220 1225 1230

Glu Leu Ala Glu Thr Ile Lys Cys Arg Thr Leu Phe Ser Thr His
1235 1240 1245

Tyr His Ser Leu Val Glu Asp Tyr Ser Gln Asn Val Ala Val Arg
1250 1255 1260

Leu Gly His Met Ala Cys Met Val Glu Asn Glu Cys Glu Asp Pro
1265 1270 1275

Ser Gln Glu Thr Ile Thr Phe Leu Tyr Lys Phe Ile Lys Gly Ala
1280 1285 1290

Cys Pro Lys Ser Tyr Gly Phe Asn Ala Ala Arg Leu Ala Asn Leu
1295 1300 1305

Pro Glu Glu Val Ile Gln Lys Gly His Arg Lys Ala Arg Glu Phe
1310 1315 1320

Glu Lys Met Asn Gln Ser Leu Arg Leu Phe Arg Glu Val Cys Leu
1325 1330 1335

Ala Ser Glu Arg Ser Thr Val Asp Ala Glu Ala Val His Lys Leu
1340 1345 1350

Leu Thr Leu Ile Lys Glu Leu
1355 1360

<210> 17
<211> 1408
<212> DNA
<213> Homo sapiens

<400> 17
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ttggggagcc agtacatgca ggtgggctcc acacggagag ggcgcagac ccggtgacag 120

ggctttacct ggtacatcgg catggcgcaa ccaaagcaag agagggtggc gcgtgccaga 180

caccaacggt cggaaaccgc cagacaccaa cggtcggaaa ccgccaagac accaacgctc 240

ggaaaccgcc agacaccaac gctcggaaac cgccagacac caaggctcgg aatccacgcc 300

aggccacgac ggagggcgac tacctccctt ctgaccctgc tgctggcgtt cggaaaaaac 360

gcagtccggt gtgctctgat tggtccaggc tctttgacgt cacggactcg acctttgaca 420

gagccactag gcgaaaagga gagacgggaa gtatttttc cgccccgccc ggaaagggtg 480

gagcacaacg tcgaaagcag ccgttgggag cccaggaggc ggggcgcctg tgggagccgt 540

ggagggaact ttcccagtcc ccgaggcgga tccggtgttg catccttgga gcgagctgag 600

aactcgagta cagaacctgc taaggccatc aaacctattg atcggaagtc agtccatcag 660

atttgctctg ggccggtggt accgagtcta aggccgaatg cggtgaagga gttagtagaa 720

aacagtctgg atgctggtgc cactaatgtt gatctaaagc ttaaggacta tggagtggat 780

ctcattgaag tttcaggcaa tggatgtggg gtagaagaag aaaacttcga aggctttact 840

ctgaaacatc acacatgtaa gattcaagag tttgccgacc taactcaggt ggaaactttt 900

ggctttcggg gggaagctct gagctcactt tgtgcactga gtgatgtcac catttctacc 960

tgccgtgtat cagcgaaggt tgggactcga ctggtgtttg atcactatgg gaaaatcatc 1020

cagaaaaccc cctacccccg ccccagaggg atgacagtca gcgtgaagca gttatttttct 1080
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cagcctgtag aactgactcc tagaagtacc ccaccccacc cctgctcctt ggaggacaac 1260
gtgatcactg tattcagctc tgtcaagaat ggtccaggtt cttctagatg atctgcacaa 1320
atggttcctc tcctccttcc tgatgtctgc cattagcatt ggaataaagt tcctgctgaa 1380
aatccaaaaa aaaaaaaaaa aaaaaaa 1408

<210> 18
<211> 389
<212> PRT
<213> Homo sapiens

<400> 18

Met Ala Gln Pro Lys Gln Glu Arg Val Ala Arg Ala Arg His Gln Arg
1 5 10 15

Ser Glu Thr Ala Arg His Gln Arg Ser Glu Thr Ala Lys Thr Pro Thr
20 25 30

Leu Gly Asn Arg Gln Thr Pro Thr Leu Gly Asn Arg Gln Thr Pro Arg
35 40 45

Leu Gly Ile His Ala Arg Pro Arg Arg Arg Ala Thr Thr Ser Leu Leu
50 55 60

Thr Leu Leu Leu Ala Phe Gly Lys Asn Ala Val Arg Cys Ala Leu Ile
65 70 75 80

Gly Pro Gly Ser Leu Thr Ser Arg Thr Arg Pro Leu Thr Glu Pro Leu
85 90 95

Gly Glu Lys Glu Arg Arg Glu Val Phe Phe Pro Pro Arg Pro Glu Arg
100 105 110

Val Glu His Asn Val Glu Ser Ser Arg Trp Glu Pro Arg Arg Arg Gly
115 120 125

Ala Cys Gly Ser Arg Gly Gly Asn Phe Pro Ser Pro Arg Gly Gly Ser
130 135 140

Gly Val Ala Ser Leu Glu Arg Ala Glu Asn Ser Ser Thr Glu Pro Ala
145 150 155 160

Lys Ala Ile Lys Pro Ile Asp Arg Lys Ser Val His Gln Ile Cys Ser

165 170 175

Gly Pro Val Val Pro Ser Leu Arg Pro Asn Ala Val Lys Glu Leu Val
180 185 190

Glu Asn Ser Leu Asp Ala Gly Ala Thr Asn Val Asp Leu Lys Leu Lys
195 200 205

Asp Tyr Gly Val Asp Leu Ile Glu Val Ser Gly Asn Gly Cys Gly Val
210 215 220

Glu Glu Glu Asn Phe Glu Gly Phe Thr Leu Lys His His Thr Cys Lys
225 230 235 240

Ile Gln Glu Phe Ala Asp Leu Thr Gln Val Glu Thr Phe Gly Phe Arg
245 250 255

Gly Glu Ala Leu Ser Ser Leu Cys Ala Leu Ser Asp Val Thr Ile Ser
260 265 270

Thr Cys Arg Val Ser Ala Lys Val Gly Thr Arg Leu Val Phe Asp His
275 280 285

Tyr Gly Lys Ile Ile Gln Lys Thr Pro Tyr Pro Arg Pro Arg Gly Met
290 295 300

Thr Val Ser Val Lys Gln Leu Phe Ser Thr Leu Pro Val His His Lys
305 310 315 320

Glu Phe Gln Arg Asn Ile Lys Lys Lys Arg Ala Cys Phe Pro Phe Ala
325 330 335

Phe Cys Arg Asp Cys Gln Phe Pro Glu Ala Ser Pro Ala Met Leu Pro
340 345 350

Val Gln Pro Val Glu Leu Thr Pro Arg Ser Thr Pro Pro His Pro Cys
355 360 365

Ser Leu Glu Asp Asn Val Ile Thr Val Phe Ser Ser Val Lys Asn Gly
370 375 380

Pro Gly Ser Ser Arg
385

<210> 19
<211> 1785
<212> DNA
<213> Homo sapiens

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gggagctgca gaccagtctt ccgtggcagg ctgagcgctc cagtcttcag tagggaattg 180
ctgaataggc acagagggca cctgtacacc ttcagaccag tctgcaacct caggctgagt 240
agcagtgaac tcaggagcgg gagcagtcca ttcaccctga aattcctcct tggtcactgc 300
cttctcagca gcagcctgct cttctttttc aatctcttca ggatctctgt agaagtacag 360
atcaggcatg acctcccatg ggtgttcacg ggaaatggtg ccacgcatgc gcagaacttc 420
ccgagccagc atccaccaca ttaaacccac tgagtgagct cccttgttgt tgcatgggat 480
ggcaatgtcc acatagcgca gaggagaatc tgtgttacac agcgcaatgg taggtaggtt 540
aacataagat gcctccgtga gaggcgaagg ggcggcggga cccgggcctg gcccgtatgt 600
gtccttggcg gcctagacta ggccgtcgct gtatggtgag ccccagggag gcggatctgg 660
gcccccagaa ggacacccgc ctggatttgc cccgtagccc ggcccgggcc cctcgggagc 720
agaacagcct tggtgaggtg gacaggaggg gacctcgcga gcagacgcgc gcgccagcga 780
cagcagcccc gccccggcct ctcgggagcc gggggcaga ggctgcggag ccccaggagg 840
gtctatcagc cacagtctct gcatgtttcc aagagcaaca ggaaatgaac acattgcagg 900
ggccagtgtc attcaaagat gtggctgtgg atttcaccca ggaggagtgg cggcaactgg 960
accctgatga gaagatagca tacggggatg tgatgttgga gaactacagc catctagttt 1020
ctgtggggta tgattatcac caagccaaac atcatcatgg agtggaggtg aaggaagtgg 1080
agcagggaga ggagccgtgg ataatggaag gtgaatttcc atgtcaacat agtccagaac 1140
ctgctaaggc catcaaacct attgatcgga agtcagtcca tcagatttgc tctgggccag 1200
tggtactgag tctaagcact gcagtgaagg agttagtaga aaacagtctg gatgctggtg 1260
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cacacatgta agattcaaga gtttgccgac ctaactgaag ttgaaacttt cggttttcag 1440
ggggaagctc tgagctcact gtgtgcactg agcgatgtca ccatttctac ctgccacgcg 1500
ttggtgaagg ttgggactcg actggtgttt gatcacgatg ggaaaatcat ccaggaaacc 1560
ccctaccccc accccagagg gaccacagtc agcgtgaagc agttattttc tacgctacct 1620
gtgcgccata aggaatttca aaggaatatt aagaagacgt gcctgcttcc ccttcgcctt 1680
ctgccgtgat tgtcagtttc ctgaggcctc cccagccatg cttcctgtac agcctgcaga 1740
actgtgagtc aattaaacct cttttcttca taaattaaaa aaaaa 1785

<210> 20
<211> 264

<212> PRT
<213> Homo sapiens

<400> 20

Met Cys Pro Trp Arg Pro Arg Leu Gly Arg Arg Cys Met Val Ser Pro
1 5 10 15

Arg Glu Ala Asp Leu Gly Pro Gln Lys Asp Thr Arg Leu Asp Leu Pro
20 25 30

Arg Ser Pro Ala Arg Ala Pro Arg Glu Gln Asn Ser Leu Gly Glu Val
35 40 45

Asp Arg Arg Gly Pro Arg Glu Gln Thr Arg Ala Pro Ala Thr Ala Ala
50 55 60

Pro Pro Arg Pro Leu Gly Ser Arg Gly Ala Glu Ala Ala Glu Pro Gln
65 70 75 80

Glu Gly Leu Ser Ala Thr Val Ser Ala Cys Phe Gln Glu Gln Gln Glu
85 90 95

Met Asn Thr Leu Gln Gly Pro Val Ser Phe Lys Asp Val Ala Val Asp
100 105 110

Phe Thr Gln Glu Glu Trp Arg Gln Leu Asp Pro Asp Glu Lys Ile Ala
115 120 125

Tyr Gly Asp Val Met Leu Glu Asn Tyr Ser His Leu Val Ser Val Gly
130 135 140

Tyr Asp Tyr His Gln Ala Lys His His His Gly Val Glu Val Lys Glu
145 150 155 160

Val Glu Gln Gly Glu Glu Pro Trp Ile Met Glu Gly Glu Phe Pro Cys
165 170 175

Gln His Ser Pro Glu Pro Ala Lys Ala Ile Lys Pro Ile Asp Arg Lys
180 185 190

Ser Val His Gln Ile Cys Ser Gly Pro Val Val Leu Ser Leu Ser Thr
195 200 205

Ala Val Lys Glu Leu Val Glu Asn Ser Leu Asp Ala Gly Ala Thr Asn
210 215 220

Ile Asp Leu Lys Leu Lys Asp Tyr Gly Val Asp Leu Ile Glu Val Ser
225 230 235 240

Asp Asn Gly Cys Gly Val Glu Glu Glu Asn Phe Glu Gly Leu Ile Ser
245 250 255

Phe Ser Ser Glu Thr Ser His Met
260

<210> 21
<211> 795
<212> DNA
<213> Homo sapiens

<400> 21
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ctgggccccc agaaggacac ccgcctggat ttgccccgta gcccggcccg ggcccctcgg 120
gagcagaaca gccttggtga ggtggacagg aggggacctc gcgagcagac gcgcgcgcca 180
gcgacagcag ccccgccccg gcctctcggg agccgggggg cagaggctgc ggagccccag 240
gagggtctat cagccacagt ctctgcatgt ttccaagagc aacaggaaat gaacacattg 300
caggggccag tgtcattcaa agatgtggct gtggatttca cccaggagga gtggcggcaa 360
ctggaccctg atgagaagat agcatacggg gatgtgatgt tggagaacta cagccatcta 420
gtttctgtgg ggtatgatta tcaccaagcc aaacatcatc atggagtgga ggtgaaggaa 480
gtggagcagg gagaggagcc gtggataatg gaaggtgaat ttccatgtca acatagtcca 540
gaacctgcta aggccatcaa acctattgat cggaagtcag tccatcagat ttgctctggg 600
ccagtggtac tgagtctaag cactgcagtg aaggagttag tagaaaacag tctggatgct 660
ggtgccacta atattgatct aaagcttaag gactatggag tggatctcat tgaagtttca 720
gacaatggat gtggggtaga agaagaaaac tttgaaggct taatctcttt cagctctgaa 780
acatcacaca tgtaa 795

<210> 22
<211> 264
<212> PRT
<213> Homo sapiens

<400> 22

Met Cys Pro Trp Arg Pro Arg Leu Gly Arg Arg Cys Met Val Ser Pro
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Arg Glu Ala Asp Leu Gly Pro Gln Lys Asp Thr Arg Leu Asp Leu Pro
20 25 30

Arg Ser Pro Ala Arg Ala Pro Arg Glu Gln Asn Ser Leu Gly Glu Val
35 40 45

Asp Arg Arg Gly Pro Arg Glu Gln Thr Arg Ala Pro Ala Thr Ala Ala

50 55 60

Pro Pro Arg Pro Leu Gly Ser Arg Gly Ala Glu Ala Ala Glu Pro Gln
65 70 75 80

Glu Gly Leu Ser Ala Thr Val Ser Ala Cys Phe Gln Glu Gln Gln Glu
85 90 95

Met Asn Thr Leu Gln Gly Pro Val Ser Phe Lys Asp Val Ala Val Asp
100 105 110

Phe Thr Gln Glu Glu Trp Arg Gln Leu Asp Pro Asp Glu Lys Ile Ala
115 120 125

Tyr Gly Asp Val Met Leu Glu Asn Tyr Ser His Leu Val Ser Val Gly
130 135 140

Tyr Asp Tyr His Gln Ala Lys His His His Gly Val Glu Val Lys Glu
145 150 155 160

Val Glu Gln Gly Glu Glu Pro Trp Ile Met Glu Gly Glu Phe Pro Cys
165 170 175

Gln His Ser Pro Glu Pro Ala Lys Ala Ile Lys Pro Ile Asp Arg Lys
180 185 190

Ser Val His Gln Ile Cys Ser Gly Pro Val Val Leu Ser Leu Ser Thr
195 200 205

Ala Val Lys Glu Leu Val Glu Asn Ser Leu Asp Ala Gly Ala Thr Asn
210 215 220

Ile Asp Leu Lys Leu Lys Asp Tyr Gly Val Asp Leu Ile Glu Val Ser
225 230 235 240

Asp Asn Gly Cys Gly Val Glu Glu Glu Asn Phe Glu Gly Leu Ile Ser
245 250 255

Phe Ser Ser Glu Thr Ser His Met
260

<210> 23
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic oligonucleotide primer

<400> 23

acgcatatgg agcgagctga gagctcgagt 30

<210> 24
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic oligonucleotide primer

<400> 24
gaattcttat cacgtagaat cgagaccgag gagagggtta gggataggct taccagttcc 60

aaccttcgcc gatgc 75

<210> 25
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic oligonucleotide primer

<400> 25
acgcatatgt gtccttggcg gcctaga 27

<210> 26
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic oligonucleotide primer

<400> 26
gaattcttat tacgtagaat cgagaccgag gagagggtta gggataggct tacccatgtg 60

tgatgtttca gagct 75

<210> 27
<211> 49
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic oligonucleotide primer

<220>
<221> misc_feature
<222> (35)..(35)
<223> corresponds to the first nucleotide of the first codon of the tar
get mismatch repair gene

<220>
<221> misc_feature
<222> (36)..(36)
<223> corresponds to the second nucleotide of the first codon of the ta
rget mismatch repair gene

<220>
<221> misc_feature
<222> (37)..(37)
<223> corresponds to the third nucleotide of the first codon of the tar get mismatch repair gene

<220>
<221> misc_feature
<222> (38)..(38)
<223> corresponds to the first nucleotide of the second codon of the ta rget mismatch repair gene

<220>
<221> misc_feature
<222> (39)..(39)
<223> corresponds to the second nucleotide of the second codon of the t arget mismatch repair gene

<220>
<221> misc_feature
<222> (40)..(40)
<223> corresponds to the third nucleotide of the second codon of the ta rget mismatch repair gene

<220>
<221> misc_feature
<222> (41)..(41)
<223> corresponds to the first nucleotide of the third codon of the tar get mismatch repair gene

<220>
<221> misc_feature
<222> (42)..(42)
<223> corresponds to the second nucleotide of the third codon of the ta rget mismatch repair gene

<220>
<221> misc_feature
<222> (43)..(43)
<223> corresponds to the third nucleotide of the third codon of the tar get mismatch repair gene

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<223> corresponds to the first nucleotide of the fourth codon of the ta rget mismatch repair gene

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<220>
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<222> (46)..(46)
<223> corresponds to the third nucleotide of the fourth codon of the ta rget mismatch repair gene

<220>
<221> misc_feature
<222> (47)..(47)
<223> corresponds to the first nucleotide of the fifth codon of the tar get mismatch repair gene

<220>
<221> misc_feature
<222> (48)..(48)
<223> corresponds to the second nucleotide of the fifth codon of the ta rget mismatch repair gene

<220>
<221> misc_feature
<222> (49)..(49)
<223> corresponds to the third nucleotide of the fifth codon of the tar get mismatch repair gene

<400> 27
tttaatacga ctcactatag ggagaccacc atggnnnnnn nnnnnnnnn 49

<210> 28
<211> 4290
<212> DNA
<213> Homo sapiens

<400> 28
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<212> PRT
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<400> 29

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Ser Ile Asp Ala Glu Ala Lys Cys Val Ala Val Arg Val Asn Met Glu
35 40 45

Thr Phe Gln Val Gln Val Ile Asp Asn Gly Phe Gly Met Gly Ser Asp
50 55 60

Asp Val Glu Lys Val Gly Asn Arg Tyr Phe Thr Ser Lys Cys His Ser
65 70 75 80

Val Gln Asp Leu Glu Asn Pro Arg Phe Tyr Gly Phe Arg Gly Glu Ala
85 90 95

Leu Ala Asn Ile Ala Asp Met Ala Ser Ala Val Glu Ile Ser Ser Lys
100 105 110

Lys Asn Arg Thr Met Lys Thr Phe Val Lys Leu Phe Gln Ser Gly Lys
115 120 125

Ala Leu Lys Ala Cys Glu Ala Asp Val Thr Arg Ala Ser Ala Gly Thr
130 135 140

Thr Val Thr Val Tyr Asn Leu Phe Tyr Gln Leu Pro Val Arg Arg Lys
145 150 155 160

Cys Met Asp Pro Arg Leu Glu Phe Glu Lys Val Arg Gln Arg Ile Glu
165 170 175

Ala Leu Ser Leu Met His Pro Ser Ile Ser Phe Ser Leu Arg Asn Asp
180 185 190

Val Ser Gly Ser Met Val Leu Gln Leu Pro Lys Thr Lys Asp Val Cys
195 200 205

Ser Arg Phe Cys Gln Ile Tyr Gly Leu Gly Lys Ser Gln Lys Leu Arg
210 215 220

Glu Ile Ser Phe Lys Tyr Lys Glu Phe Glu Leu Ser Gly Tyr Ile Ser
225 230 235 240

Ser Glu Ala His Tyr Asn Lys Asn Met Gln Phe Leu Phe Val Asn Lys
245 250 255

Arg Leu Val Leu Arg Thr Lys Leu His Lys Leu Ile Asp Phe Leu Leu
260 265 270

Arg Lys Glu Ser Ile Ile Cys Lys Pro Lys Asn Gly Pro Thr Ser Arg
275 280 285

Gln Met Asn Ser Ser Leu Arg His Arg Ser Thr Pro Glu Leu Tyr Gly
290 295 300

Ile Tyr Val Ile Asn Val Gln Cys Gln Phe Cys Glu Tyr Asp Val Cys
305 310 315 320

Met Glu Pro Ala Lys Thr Leu Ile Glu Phe Gln Asn Trp Asp Thr Leu
325 330 335

Leu Phe Cys Ile Gln Glu Gly Val Lys Met Phe Leu Lys Gln Glu Lys
340 345 350

Leu Phe Val Glu Leu Ser Gly Glu Asp Ile Lys Glu Phe Ser Glu Asp
355 360 365

Asn Gly Phe Ser Leu Phe Asp Ala Thr Leu Gln Lys Arg Val Thr Ser
370 375 380

Asp Glu Arg Ser Asn Phe Gln Glu Ala Cys Asn Asn Ile Leu Asp Ser
385 390 395 400

Tyr Glu Met Phe Asn Leu Gln Ser Lys Ala Val Lys Arg Lys Thr Thr
405 410 415

Ala Glu Asn Val Asn Thr Gln Ser Ser Arg Asp Ser Glu Ala Thr Arg
420 425 430

Lys Asn Thr Asn Asp Ala Phe Leu Tyr Ile Tyr Glu Ser Gly Gly Pro
435 440 445

Gly His Ser Lys Met Thr Glu Pro Ser Leu Gln Asn Lys Asp Ser Ser
450 455 460

Cys Ser Glu Ser Lys Met Leu Glu Gln Glu Thr Ile Val Ala Ser Glu
465 470 475 480

Ala Gly Glu Asn Glu Lys His Lys Lys Ser Phe Leu Glu His Ser Ser
485 490 495

Leu Glu Asn Pro Cys Gly Thr Ser Leu Glu Met Phe Leu Ser Pro Phe
500 505 510

Gln Thr Pro Cys His Phe Glu Glu Ser Gly Gln Asp Leu Glu Ile Trp
515 520 525

Lys Glu Ser Thr Thr Val Asn Gly Met Ala Ala Asn Ile Leu Lys Asn
530 535 540

Asn Arg Ile Gln Asn Gln Pro Lys Arg Phe Lys Asp Ala Thr Glu Val
545 550 555 560

Gly Cys Gln Pro Leu Pro Phe Ala Thr Thr Leu Trp Gly Val His Ser
565 570 575

Ala Gln Thr Glu Lys Glu Lys Lys Lys Glu Ser Ser Asn Cys Gly Arg
580 585 590

Arg Asn Val Phe Ser Tyr Gly Arg Val Lys Leu Cys Ser Thr Gly Phe
595 600 605

Ile Thr His Val Val Gln Asn Glu Lys Thr Lys Ser Thr Glu Thr Glu
610 615 620

His Ser Phe Lys Asn Tyr Val Arg Pro Gly Pro Thr Arg Ala Gln Glu
625 630 635 640

Thr Phe Gly Asn Arg Thr Arg His Ser Val Glu Thr Pro Asp Ile Lys
645 650 655

Asp Leu Ala Ser Thr Leu Ser Lys Glu Ser Gly Gln Leu Pro Asn Lys
660 665 670

Lys Asn Cys Arg Thr Asn Ile Ser Tyr Gly Leu Glu Asn Glu Pro Thr
675 680 685

Ala Thr Tyr Thr Met Phe Ser Ala Phe Gln Glu Gly Ser Lys Lys Ser
690 695 700

Gln Thr Asp Cys Ile Leu Ser Asp Thr Ser Pro Ser Phe Pro Trp Tyr
705 710 715 720

Arg His Val Ser Asn Asp Ser Arg Lys Thr Asp Lys Leu Ile Gly Phe
725 730 735

Ser Lys Pro Ile Val Arg Lys Lys Leu Ser Leu Ser Ser Gln Leu Gly
740 745 750

Ser Leu Glu Lys Phe Lys Arg Gln Tyr Gly Lys Val Glu Asn Pro Leu
755 760 765

Asp Thr Glu Val Glu Glu Ser Asn Gly Val Thr Thr Asn Leu Ser Leu
770 775 780

Gln Val Glu Pro Asp Ile Leu Leu Lys Asp Lys Asn Arg Leu Glu Asn
785 790 795 800

Ser Asp Val Cys Lys Ile Thr Thr Met Glu His Ser Asp Ser Asp Ser
805 810 815

Ser Cys Gln Pro Ala Ser His Ile Leu Asp Ser Glu Lys Phe Pro Phe
820 825 830

Ser Lys Asp Glu Asp Cys Leu Glu Gln Gln Met Leu Ser Leu Arg Glu
835 840 845

Ser Pro Met Thr Leu Lys Glu Leu Ser Leu Phe Asn Arg Lys Pro Leu
850 855 860

Asp Leu Glu Lys Ser Ser Glu Ser Leu Ala Ser Lys Leu Ser Arg Leu
865 870 875 880

Lys Gly Ser Glu Arg Glu Thr Gln Thr Met Gly Met Met Ser Arg Phe
885 890 895

Asn Glu Leu Pro Asn Ser Asp Ser Ser Arg Lys Asp Ser Lys Leu Cys
900 905 910

Ser Val Leu Thr Gln Asp Phe Cys Met Leu Phe Asn Asn Lys His Glu
915 920 925

Lys Thr Glu Asn Gly Val Ile Pro Thr Ser Asp Ser Ala Thr Gln Asp
930 935 940

Asn Ser Phe Asn Lys Asn Ser Lys Thr His Ser Asn Ser Asn Thr Thr
945 950 955 960

Glu Asn Cys Val Ile Ser Glu Thr Pro Leu Val Leu Pro Tyr Asn Asn
965 970 975

Ser Lys Val Thr Gly Lys Asp Ser Asp Val Leu Ile Arg Ala Ser Glu
980 985 990

Gln Gln Ile Gly Ser Leu Asp Ser Pro Ser Gly Met Leu Met Asn Pro
995 1000 1005

Val Glu Asp Ala Thr Gly Asp Gln Asn Gly Ile Cys Phe Gln Ser
1010 1015 1020

Glu Glu Ser Lys Ala Arg Ala Cys Ser Glu Thr Glu Glu Ser Asn
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Thr Cys Cys Ser Asp Trp Gln Arg His Phe Asp Val Ala Leu Gly
1040 1045 1050

Arg Met Val Tyr Val Asn Lys Met Thr Gly Leu Ser Thr Phe Ile
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Ala Pro Thr Glu Asp Ile Gln Ala Ala Cys Thr Lys Asp Leu Thr
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Thr Val Ala Val Asp Val Val Leu Glu Asn Gly Ser Gln Tyr Arg
1085 1090 1095

Cys Gln Pro Phe Arg Ser Asp Leu Val Leu Pro Phe Leu Pro Arg
1100 1105 1110

Ala Arg Ala Glu Arg Thr Val Met Arg Gln Asp Asn Arg Asp Thr
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Val Asp Asp Thr Val Ser Ser Glu Ser Leu Gln Ser Leu Phe Ser
1130 1135 1140

Glu Trp Asp Asn Pro Val Phe Ala Arg Tyr Pro Glu Val Ala Val
1145 1150 1155

Asp Val Ser Ser Gly Gln Ala Glu Ser Leu Ala Val Lys Ile His
1160 1165 1170

Asn Ile Leu Tyr Pro Tyr Arg Phe Thr Lys Gly Met Ile His Ser
1175 1180 1185

Met Gln Val Leu Gln Gln Val Asp Asn Lys Phe Ile Ala Cys Leu
1190 1195 1200

Met Ser Thr Lys Thr Glu Glu Asn Gly Glu Ala Asp Ser Tyr Glu
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Lys Gln Gln Ala Gln Gly Ser Gly Arg Lys Lys Leu Leu Ser Ser
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Thr Leu Ile Pro Pro Leu Glu Ile Thr Val Thr Glu Glu Gln Arg
1235 1240 1245

Arg Leu Leu Trp Cys Tyr His Lys Asn Leu Glu Asp Leu Gly Leu
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Glu Phe Val Phe Pro Asp Thr Ser Asp Ser Leu Val Leu Val Gly
1265 1270 1275

Lys Val Pro Leu Cys Phe Val Glu Arg Glu Ala Asn Glu Leu Arg
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Arg Gly Arg Ser Thr Val Thr Lys Ser Ile Val Glu Glu Phe Ile
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Arg Glu Gln Leu Glu Leu Leu Gln Thr Thr Gly Gly Ile Gln Gly
1310 1315 1320

Thr Leu Pro Leu Thr Val Gln Lys Val Leu Ala Ser Gln Ala Cys
1325 1330 1335

His Gly Ala Ile Lys Phe Asn Asp Gly Leu Ser Leu Gln Glu Ser
1340 1345 1350

Cys Arg Leu Ile Glu Ala Leu Ser Ser Cys Gln Leu Pro Phe Gln
1355 1360 1365

Cys Ala His Gly Arg Pro Ser Met Leu Pro Leu Ala Asp Ile Asp
1370 1375 1380

His Leu Glu Gln Glu Lys Gln Ile Lys Pro Asn Leu Thr Lys Leu
1385 1390 1395

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Asp Thr Arg Gln Ser Leu Gln Gln Ser Met Pro Pro Cys Glu Pro
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<212> PRT
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<400> 31

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Val Ile Leu Asp Leu Ser Ser Ala Val Lys Glu Leu Val Glu Asn Ser
35 40 45

Leu Asp Ala Gly Ala Thr Ser Ile Glu Ile Asn Leu Arg Asp Tyr Gly
50 55 60

Glu Asp Tyr Phe Gln Val Ile Asp Asn Gly Cys Gly Ile Ser Pro Thr
65 70 75 80

Asn Phe Lys Val Cys Val Gln Ile Leu Arg Arg Thr Phe Asp Val Leu
85 90 95

Ala Leu Lys His His Thr Ser Lys Leu Glu Asp Phe Thr Asp Leu Leu
100 105 110

Asn Leu Thr Thr Tyr Gly Phe Arg Gly Glu Ala Leu Ser Ser Leu Cys
115 120 125

Ala Leu Gly Asn Leu Thr Val Glu Thr Arg Thr Lys Asn Glu Pro Val
130 135 140

Ala Thr Leu Leu Thr Phe Asp His Ser Gly Leu Leu Thr Ala Glu Lys
145 150 155 160

Lys Thr Ala Arg Gln Ile Gly Thr Thr Val Thr Val Arg Lys Leu Phe
165 170 175

Ser Asn Leu Pro Val Arg Ser Lys Glu Phe Lys Arg Asn Ile Arg Lys
180 185 190

Glu Tyr Gly Lys Leu Val Ser Leu Leu Asn Ala Tyr Ala Leu Ile Ala
195 200 205

Lys Gly Val Arg Phe Val Cys Ser Asn Thr Thr Gly Lys Asn Pro Lys
210 215 220

Ser Val Val Leu Asn Thr Gln Gly Arg Gly Ser Leu Lys Asp Asn Ile
225 230 235 240

Ile Thr Val Phe Gly Ile Ser Thr Phe Thr Ser Leu Gln Pro Gly Thr
245 250 255

Gly Arg Asn Leu Ala Asp Arg Gln Tyr Phe Phe Ile Asn Gly Arg Pro
260 265 270

Val Asp Met Pro Lys Val Ser Lys Leu Val Asn Glu Leu Tyr Lys Asp
275 280 285

Thr Ser Ser Arg Lys Tyr Pro Val Thr Ile Leu Asp Phe Ile Val Pro
290 295 300

Gly Gly Ala Cys Asp Leu Asn Val Thr Pro Asp Lys Arg Lys Val Phe
305 310 315 320

Phe Ser Asp Glu Thr Ser Val Ile Gly Ser Leu Arg Glu Gly Leu Asn
325 330 335

Glu Ile Tyr Ser Ser Ser Asn Ala Ser Tyr Ile Val Asn Arg Phe Glu
340 345 350

Glu Asn Ser Glu Gln Pro Asp Lys Ala Gly Val Ser Ser Phe Gln Lys
355 360 365

Lys Ser Asn Leu Leu Ser Glu Gly Ile Val Leu Asp Val Ser Ser Lys
370 375 380

Thr Arg Leu Gly Glu Ala Ile Glu Lys Glu Asn Pro Ser Leu Arg Glu
385 390 395 400

Val Glu Ile Asp Asn Ser Ser Pro Met Glu Lys Phe Lys Phe Glu Ile
405 410 415

Lys Ala Cys Gly Thr Lys Lys Gly Glu Gly Ser Leu Ser Val His Asp
420 425 430

Val Thr His Leu Asp Lys Thr Pro Ser Lys Gly Leu Pro Gln Leu Asn
435 440 445

Val Thr Glu Lys Val Thr Asp Ala Ser Lys Asp Leu Ser Ser Arg Ser
450 455 460

Ser Phe Ala Gln Ser Thr Leu Asn Thr Phe Val Thr Met Gly Lys Arg
465 470 475 480

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Asn Gln Thr Ser Ser Tyr Arg Val Glu Lys Ser Lys Phe Glu Val Arg
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Ala Leu Ala Ser Arg Cys Leu Val Glu Gly Asp Gln Leu Asp Asp Met
        515                 520                 525

Val Ile Ser Lys Glu Asp Met Thr Pro Ser Glu Arg Asp Ser Glu Leu
    530                 535                 540

Gly Asn Arg Ile Ser Pro Gly Thr Gln Ala Asp Asn Val Glu Arg His
545                 550                 555                 560

Glu Arg Val Leu Gly Gln Phe Asn Leu Gly Phe Ile Ile Ala Lys Leu
                565                 570                 575

Glu Arg Asp Leu Phe Ile Val Asp Gln His Ala Ala Asp Glu Lys Phe
            580                 585                 590

Asn Phe Glu His Leu Ala Arg Ser Thr Val Leu Asn Gln Gln Pro Leu
        595                 600                 605

Leu Gln Pro Leu Asn Leu Glu Leu Ser Pro Glu Glu Glu Val Thr Val
    610                 615                 620

Leu Met His Met Asp Ile Ile Arg Glu Asn Gly Phe Leu Leu Glu Glu
625                 630                 635                 640

Asn Pro Ser Ala Pro Pro Gly Lys His Phe Arg Leu Arg Ala Ile Pro
                645                 650                 655

Tyr Ser Lys Asn Ile Thr Phe Gly Val Glu Asp Leu Lys Asp Leu Ile
            660                 665                 670

Ser Thr Leu Gly Asp Asn His Gly Glu Cys Ser Val Ala Ser Ser Tyr
        675                 680                 685

Lys Thr Ser Lys Thr Asp Ser Ile Cys Pro Ser Arg Val Arg Ala Met
    690                 695                 700

Leu Ala Ser Arg Ala Cys Arg Ser Ser Val Met Ile Gly Asp Pro Leu
705                 710                 715                 720

Arg Lys Asn Glu Met Gln Lys Ile Val Glu His Leu Ala Asp Leu Glu
                725                 730                 735
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Ser Pro Trp Asn Cys Pro His Gly Arg Pro Thr Met Arg His Leu Val
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Asp Leu Thr Thr Leu Leu Thr Leu Pro Asp Asp Asp Asn Val Asn Asp
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Asp Asp Asp Asp Asp Ala Thr Ile Ser Leu Ala
770 775

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<212> DNA
<213> Arabidopsis thaliana

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Tyr Asp His Ile Gln Glu Thr Glu Lys Arg Leu Ser Ser Ala Ser Asp
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Glu Arg Ser Arg Glu Asp Val Val Pro Leu Asn Asp Ser Ser Leu Cys
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Val Glu His Asn Val Glu Ser Ser Arg Trp Glu Pro Arg Arg Arg Gly
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325 330 335

Phe Cys Arg Asp Cys Gln Phe Pro Glu Ala Ser Pro Ala Met Leu Pro
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Pro Pro Arg Pro Leu Gly Ser Arg Gly Ala Glu Ala Ala Glu Pro Gln
65                  70                  75                  80

Glu Gly Leu Ser Ala Thr Val Ser Ala Cys Phe Gln Glu Gln Gln Glu
                85                  90                  95

Met Asn Thr Leu Gln Gly Pro Val Ser Phe Lys Asp Val Ala Val Asp
            100                 105                 110

Phe Thr Gln Glu Glu Trp Arg Gln Leu Asp Pro Asp Glu Lys Ile Ala
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Tyr Gly Asp Val Met Leu Glu Asn Tyr Ser His Leu Val Ser Val Gly
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Tyr Asp Tyr His Gln Ala Lys His His His Gly Val Glu Val Lys Glu
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Val Glu Gln Gly Glu Glu Pro Trp Ile Met Glu Gly Glu Phe Pro Cys
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Gln His Ser Pro Glu Pro Ala Lys Ala Ile Lys Pro Ile Asp Arg Lys
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Ser Val His Gln Ile Cys Ser Gly Pro Val Val Leu Ser Leu Ser Thr
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Ala Val Lys Glu Leu Val Glu Asn Ser Leu Asp Ala Gly Ala Thr Asn
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Ile Asp Leu Lys Leu Lys Asp Tyr Gly Val Asp Leu Ile Glu Val Ser
225                 230                 235                 240

Asp Asn Gly Cys Gly Val Glu Glu Glu Asn Phe Glu Gly Leu Ile Ser
                245                 250                 255

Phe Ser Ser Glu Thr Ser His Met
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/23888

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12Q 1/02, 1/68; C12N 15/00, 15/74, 1/00, 1/20
US CL :435/6, 29, 243, 252.3, 440, 471
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 29, 243, 252.3, 440, 471

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,079,154 A (ITO et al) 07 January 1992 (07.01.92), see the entire document.	1-41
A	WO 98/48041 A2 (HAKENBECK et al) 29 October 1998 (29.10.98), see the Abstract and Figures.	1-41

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 28 SEPTEMBER 2001	Date of mailing of the international search report 19 NOV 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Joyce Bridgers for GERALD GEORGE LEFFERS JR.
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

EAST: DERWENT, EPO, JPO, US-PGPUB, USPAT; STN: MEDLINE, EMBASE, BIOSIS, CAPLUS
terms: (amp, kan, tet, chl, antibiotic) resistan$2, producing, selecting, identifying, screening, obtaining, mismatch repair, proofread$3, inhibit$4, block$4